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Journal

Journal of Veterinary Diagnostic Investigation, 34(3)

ISSN

1040-6387

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Publication Date

2022-05-01

DOI

10.1177/10406387211057469

Peer reviewed

Journal of Veterinary Diagnostic Investigation
2022, Vol. 34(3) 354–375

DOI: 10.1177/10406387211057469
jvdi.sagepub.com

Bacterial and viral enterocolitis in horses: a review

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Abstract. Enteritis, colitis, and enterocolitis are considered some of the most common causes of disease and death in horses. Determining the etiology of these conditions is challenging, among other reasons because different causes produce similar clinical signs and lesions, and also because some agents of colitis can be present in the intestine of normal animals. We review here the main bacterial and viral causes of enterocolitis of horses, including *Salmonella* spp., *Clostridium perfringens* type A NetF-positive, *C. perfringens* type C, *Clostridioides difficile*, *Clostridium piliforme*, *Paeniclostridium sordellii*, other clostridia, *Rhodococcus equi*, *Neorickettsia risticii*, *Lawsonia intracellularis*, equine rotavirus, and equine coronavirus. Diarrhea and colic are the hallmark clinical signs of colitis and enterocolitis, and the majority of these conditions are characterized by necrotizing changes in the mucosa of the small intestine, colon, cecum, or in a combination of these organs. The presumptive diagnosis is based on clinical, gross, and microscopic findings, and confirmed by detection of some of the agents and/or their toxins in the intestinal content or feces.

Keywords: colitis; enteritis; enterocolitis; horses; review.

Enteritis, colitis, and/or enterocolitis are among the most common causes of disease and death in horses.[79] Colic, one of the most common clinical sign of colitis and enterocolitis,[66,76] is considered the second most common cause of death of horses, after old age. Colic is estimated to have an incidence of 4.2 events/100 horses/y in the United States.[141]

The etiologic diagnosis of enteritis, colitis, and enterocolitis is challenging, with the cause undetermined in ~50% of cases.[146] The main difficulty in establishing the etiology of enterocolitis is that different causes produce similar clinical signs, and gross and microscopic lesions.[79] Additionally, several of the pathogens associated with enterocolitis can be found in the intestine of clinically healthy horses.[5,6,21,24,27,46,47,144]

Most cases of enterocolitis in horses are infectious in origin, although a few non-infectious inflammatory conditions also occur. We review here some of the most common causes of bacterial and viral enteritis, colitis, and/or enterocolitis in horses. Because many aspects of the clinical presentation, prevention, and control are similar for several of these conditions, these are discussed together for all diseases at the beginning (clinical presentation) and at the end (prevention and control) of this review. Table 1 summarizes the main epidemiologic, clinical, and pathologic characteristics of the most prevalent forms of enterocolitis in horses.

General clinical presentation of bacterial and viral enterocolitis in horses

Horses with bacterial or viral enteritis, colitis, and/or enterocolitis share similar clinical signs regardless of the etiologic agent or cause. Diarrhea, hemorrhagic or not (Fig. 1; Table 1), is the hallmark clinical sign of colitis, which leads to dehydration, electrolyte and acid-base abnormalities, most commonly hyponatremia, hypochloremia, hypokalemia, hyperlactatemia, and metabolic acidosis.[52] Fever, depression, inappetence, toxemia, colic, and leukopenia are also common clinical findings. Protein-losing enteropathy, usually characterized by hypoalbuminemia, develops frequently. On abdominal ultrasonographic examination, distended loops of small intestine and liquid content in the large colon and cecum can be observed (Fig. 2). Although the statements presented above can be applied to most cases of enterocolitis, some particular considerations regarding specific pathogens are discussed under each of the diseases presented in our review.

Bacterial pathogens

Salmonella spp.

Clinical presentation. Horses infected with *Salmonella* spp. can be clinically healthy, whereas others display mild-to-severe clinical signs. The reasons for the different clinical

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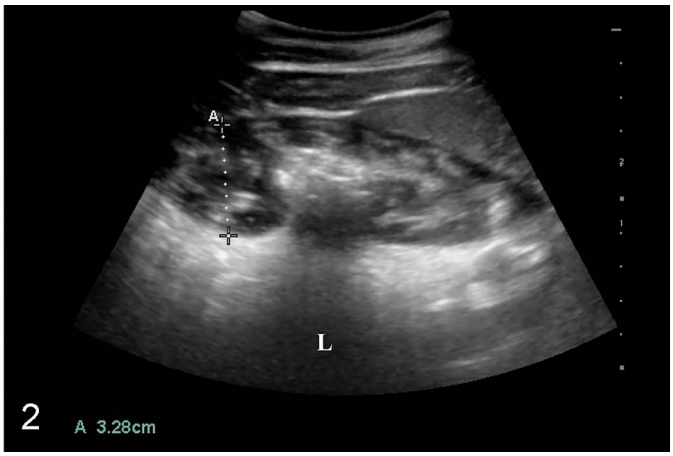
Table 1. Main clinical, epidemiologic, and pathologic characteristic of the most important causes of enterocolitis in horses.

Etiology class	Etiology	Main age affected	Main clinical signs	Main lesions	Antemortem diagnosis	Postmortem diagnosis
Bacterial	*Salmonella* spp.	All ages	Fever, diarrhea, colic	Necrotizing typhlocolitis	Clinical signs, PCR/culture of feces	Lesions, PCR/culture of intestinal content
	Clostridium perfringens type A NetF positive	Foals	Diarrhea, colic	Necrotizing enterotyphlocolitis	Not defined, isolation of NetF+ *C. perfringens* type A from feces helps but does not confirm	Not defined, isolation of NetF+ *C. perfringens* type A from intestinal content helps but does not confirm
	Clostridium perfringens type C	Neonates	Fever, diarrhea, colic, sudden death	Necrotizing enterotyphlocolitis	Clinical signs, Beta toxin detection in feces	Lesions, Beta toxin detection in intestinal content
	Clostridioides difficile	All ages	Diarrhea, colic	Necrotizing enterotyphlocolitis	Clinical signs, detection of toxins A and/or B in feces	Lesions, detection of toxins A and/or B in intestinal content
	Clostridium piliforme	Young foals	Sudden death, jaundice, lethargy	Necrotizing hepatitis, necrotizing colitis	Clinical signs	Lesions, PCR for *C. piliforme*
	Paeniclostridium sordellii	All ages	Colitis	Necrohemorrhagic enterotyphlocolitis	Clinical signs, ruling out other causes of disease, detection of *P. sordellii* by culture or PCR in feces	Lesions, ruling out other causes of disease, detection of *P. sordellii* by culture or PCR in intestinal content, detection of *P. sordellii* by immunohistochemistry in intestinal tissues
	Rhodococcus equi	Foals	Diarrhea, colic	Pyogranulomatous and ulcerative typhlocolitis	Clinical signs, isolation/PCR detection of *R. equi* in feces	Lesions, isolation/PCR detection of *R. equi* on intestinal tissue
	Neorickettsia risticii and others	All ages	Diarrhea, fever	Necrotizing typhlocolitis	Clinical signs, detection of *Neorickettsia spp.* in feces and/or peripheral blood buffy coat or whole blood	Lesions, detection of *Neorickettsia spp.* in feces and/or intestinal content
	Lawsonia intracellularis	Foals	Lethargy, anorexia, fever, peripheral edema, weight loss	Hyperplastic enteritis	Clinical signs, detection of *L. intracellularis* in feces by PCR	Lesions, detection of *L. intracellularis* in feces and/or intestinal content by PCR, detection of *L. intracellularis* in intestinal tissues by immunohistochemistry
Viral	Rotavirus	Foals	Diarrhea	Liquid content in small intestine, villus blunting	Clinical signs, detection of rotavirus in feces by PCR, ELISA, latex agglutination, electron microscopy	Lesions, detection of rotavirus in feces by PCR, ELISA, latex agglutination, electron microscopy, detection of rotavirus in intestinal tissues by immunohistochemistry
	Coronavirus	Adult horses	Inappetence, lethargy, fever	Necrotizing enteritis	Clinical signs, detection of coronavirus in feces by PCR, electron microscopy	Lesions, detection of coronavirus in feces and/or intestinal content by PCR, electron microscopy, detection of coronavirus in intestinal tissues by immunohistochemistry



Figures 1–2. Clinical manifestations of colitis in a horse. **Figure 1.** Hemorrhagic diarrhea. **Figure 2.** Ultrasound of a horse with colitis. Notice thickened and edematous colonic wall and liquid content (L). The dotted line between A and + indicates the thickness of the colonic wall (3.28 cm).

presentations include factors related to the host (e.g., stress, immune status, concurrent gastrointestinal disease) and pathogen-related factors (e.g., serotype, infecting dose). Horses with mild disease can have mild fever, soft manure, and transient reduction in feed intake. Animals with severe disease have bouts of voluminous watery diarrhea, are usually febrile, toxemic, inappetent, and may display signs of colic. Severe dehydration, electrolyte and acid-base disorders occur as a result of malabsorptive and hypersecretory diarrhea. Protein-losing enteropathy occurs frequently.[123]

Etiology. Historically, salmonellosis in horses has been associated most commonly with *Salmonella enterica* subspecies *enterica* serovar Typhimurium.[146] Several other common serovars associated with clinical enteric disease in horses include Anatum, Newport, and Angona.[31]

Epidemiology. Identifying the source of infection in individual cases and in outbreaks of salmonellosis is often difficult unless an active surveillance program for infection is in place.[20,58] Retrospective determination of the source of infection is challenging. *Salmonella* spp. are ubiquitous in the environment; transmission is fecal-oral and occurs predominantly via ingestion of feed and water contaminated by feces.[58] Direct contact with contaminated feces, environmental surfaces, feed, equipment, and caretaker hands are additional sources of exposure.[58,117] A common source of *Salmonella* spp. can be the fecal material of clinically normal shedders.[127] Horses appear to shed the bacterium more often in summer, and shedding can last for 1–4 mo, although shedding up to 14 mo has been reported.[126] *Salmonella* spp. can survive in the environment for months to years, depending on the serotype, humidity, and temperature conditions. Freezing decreases the load of the organism, but *Salmonella* spp. can be viable and infective for weeks to months under freezing conditions.[54]

The prevalence of clinical salmonellosis in the general equine population is unknown. A cross-sectional study of horses on 972 premises from 28 U.S. states in 1998 documented a prevalence of fecal shedding by normal horses of 0.8%, with 16 different serotypes identified.[140] In asymptomatic horses admitted to teaching hospitals, the prevalence of *Salmonella* spp. shedding was 1–17%.[58] The incidence of shedding was higher in horses admitted for systemic or gastrointestinal diseases (35–60%) compared to horses that were presented for lameness evaluation (15%).[21,26]

Horses of any age can be affected, although enteric forms of salmonellosis are more common in older horses. Foals and young horses are more likely to develop systemic salmonellosis than adult animals, and outbreaks can occur.[53,58] Hospitalization and associated medical (e.g., anthelmintic and antimicrobial therapy) and surgical (e.g., colic surgery, general anesthesia) procedures increase the likelihood of shedding *Salmonella* spp. in hospitals. Other risk factors associated with clinical salmonellosis include transportation, poor sanitation, overcrowding (e.g., hospitals, breeding farms), parasitic infection, foaling, colic, and diarrhea.[19,21,58]

Pathogenesis and immune response. Successful colonization, attachment, and invasion of the intestinal mucosa by the bacterium are necessary to produce disease. *Salmonella* spp. infections typically occur as a result of fecal-oral inoculation, and a relatively large number of organisms (minimum infective doses of 10^7–10^9 cfu) is necessary to produce septicemia.[8] Following ingestion, the bacterium must evade a number of nonspecific host defenses to reach the intestine. In cases of enterocolitic salmonellosis in horses, bacteria typically do not spread beyond the intestinal mucosa or local lymph nodes.[144] Pathogenic strains of *Salmonella* spp. have a number of virulence factors, often located on the so-called salmonella pathogenicity islands, that facilitate their ability to invade and survive within cells.[8] The inflammatory

response to *Salmonella* spp. infections is predominantly neutrophilic. However, the ability to survive and replicate within macrophages is necessary for *Salmonella* spp. to produce septicemia.[131]

Gross pathology. Acute cases of salmonellosis are characterized by diffuse fibrinohemorrhagic to necrotizing typhlocolitis[146] (Fig. 3). In chronic cases, enteric lesions are characterized by fibrinous or ulcerative lesions, predominantly in the cecum and colon. In some cases, transmural edema and/or button ulcers may be present. Septicemia associated with *Salmonella* spp. infection occurs more commonly in foals, and gross findings often include mucosal and serosal petechiae, pulmonary congestion and edema, and enlarged hemorrhagic lymph nodes.[144] Although gross lesions are predominantly observed in the large intestine, lesions can be seen occasionally in the small intestine.[67]

Microscopic pathology. Histologic findings of enteric salmonellosis are characterized by coagulative necrosis of the superficial mucosa, which is often covered by a fibrinocellular pseudomembrane.[67,144] Fibrin thrombi are often present in small blood vessels within the lamina propria and, sometimes, the submucosa[144] (Figs. 4, 5). As with gross changes, microscopic lesions are predominantly noted in the large intestine, but can occasionally also be observed in the small intestine.[67] In cases of septicemic salmonellosis, lesions consistent with endotoxemia may also be noted in the lung, liver, kidney, spleen, and/or adrenal gland.[67]

Diagnosis. A presumptive diagnosis of salmonellosis can often be made based on the combination of clinical signs, and gross and microscopic findings. Definitive diagnosis requires, in addition to clinical signs, gross and microscopic findings, the detection of *Salmonella* spp. in the feces, intestinal contents, and/or liver of animals with compatible clinical signs, and/or gross and microscopic lesions, by culture or PCR assay.[146] It is worth noting that *Salmonella* spp. can be isolated from the feces and intestinal contents of clinically normal horses, therefore detection of this microorganism from horses with no clinical signs or compatible lesions does not confirm a diagnosis of salmonellosis.

Clostridium perfringens

Clostridium perfringens is a gram-positive, anaerobic rod, and an important cause of enteric disease in animals. This species is divided into 7 types (A–G) on the basis of 6 major toxin genes (Table 2).[116]

Clostridium perfringens type A NetF-positive

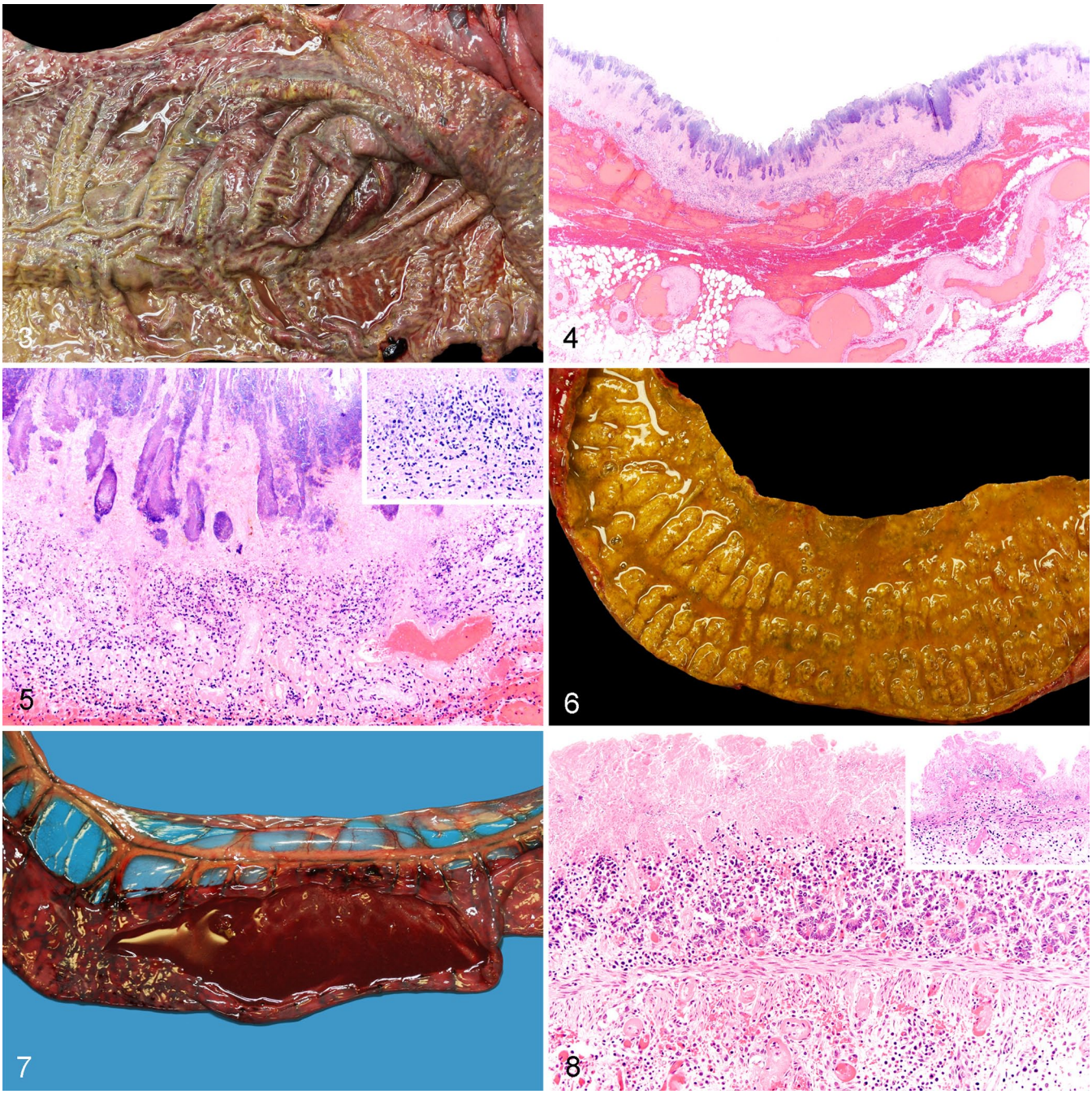
Clinical aspects. C. perfringens type A NetF-positive strains have been isolated from foals with necrotizing enterocolitis, and hence it has been postulated that they are associated with this disease, which has high mortality in neonatal foals, usually within the first 5 d of life. The most common clinical signs include anorexia, fever, colic, and hemorrhagic diarrhea. Affected foals have peracute signs, and, without aggressive supportive care, the recovery rate is very low.[87] However, *C. perfringens* type A NetF-positive strains have been isolated from healthy neonatal foals (authors' unpublished observation).

Etiology. The association between *C. perfringens* type A and enteric disease has not been confirmed definitively. The main problem with establishing a causative link between *C. perfringens* type A and enteric disease is that this microorganism can be found in the intestinal content of most healthy horses. The recent description of a novel pore-forming toxin, NetF, produced by some type A strains, has shed some light on the possible role of *netF*-positive type A isolates in enteric disease of foals.[88] NetF toxin is a member of the beta-pore-forming toxin leukocidin-hemolysin family, which is an important group of clostridial necrotizing toxins. To date, the only evidence supporting a role of NetF in enteric diseases of horses is epidemiologic and based on a higher prevalence of *netF*-positive strains in the intestine of horses with enteric disease than in healthy horses.[86,88] Attempts to reproduce the disease experimentally in laboratory animal models have been unsuccessful (authors' unpublished observation).

Epidemiology. The disease has been reported only in foals. The association between *C. perfringens netF*-positive strains and enteric disease of horses is based mostly on isolation of these strains from individual cases. The largest case series reported found *netF*-positive *C. perfringens* strains by PCR assay in 6 of 23 foals with necrotizing enteritis; these strains also carried the *C. perfringens* enterotoxin (*cpe*) gene.[87] A field investigation found no *netF*-positive strains in 137 fecal samples collected from 88 healthy foals in Ontario, Canada.[44] The trypsin-inhibiting action of mare's colostrum has been proposed as a risk factor in the development of *netF*-positive *C. perfringens*–associated enterocolitis, but remains to be proven.[87]

Pathogenesis and immune response. Very little is known about the pathogenesis of *netF*-positive *C. perfringens*–positive strain infections. However, NetF is a pore-forming toxin, and the lesions of necrotic enteritis in foals infected with these strains are very similar to those observed in animals infected with *C. perfringens* type C, whose main virulence factor is beta toxin, another pore-forming toxin. It is therefore possible that the pathogenesis of these 2 infections is similar.

Gross pathology. The gross pathology of *netF*-positive *C. perfringens* type A–associated disease is characterized



Figures 3–8. Equine enterocolitis of bacterial etiology. **Figure 3.** Colitis produced by *Salmonella enterica* serovar Typhimurium in a horse. The mucosa is hemorrhagic, necrotic, and covered by a fibrinous pseudomembrane. Photo courtesy of Dr. Francisco Carvallo. **Figure 4.** Colitis produced by *S. enterica* serovar Typhimurium in a horse. The mucosa is diffusely necrotic and there is transmural hemorrhage. H&E. **Figure 5.** Colitis produced by *S. enterica* serovar Typhimurium in a horse. There is a diffuse inflammatory infiltrate of the deep lamina propria. Inset: higher magnification showing large number of neutrophils in the lamina propria. H&E. **Figure 6.** Necrotic enteritis in a foal associated with NetF-positive *Clostridium perfringens* type A. Reproduced with permission from Mehdizadeh Gohari et al.[88] **Figure 7.** Necrotic enteritis produced by *C. perfringens* type C in a neonatal foal. There is transmural hemorrhage and hemorrhagic content within the lumen. **Figure 8.** Necrotic enteritis produced by *C. perfringens* type C in a neonatal foal. There is severe and diffuse mucosal necrosis. Inset: higher magnification of the lamina propria and submucosal thrombosis. H&E.

by fibrinonecrotic enteritis or enterocolitis. A fibrinous pseudomembrane is usually present covering the small and/or large intestinal mucosa (Fig. 6).[88]

Microscopic pathology. The mucosa of the small intestine has undergone coagulative necrosis, which is separated from the underlying, more normal-appearing intestinal tissue by a

Table 2. Toxinotypes of *Clostridium perfringens* (modified from Rood et al.[116]).

	Toxin produced					
Toxinotype	CPA	CPB	ETX	ITX	CPE	NetB
A	+	−	−	−	−	−
B	+	+	+	−	−	−
C	+	+	−	−	±	−
D	+	−	+	−	±	−
E	+	−	−	+	±	−
F	+	−	−	−	+	−
G	+	−	−	−	−	+

CPA = alpha; CPB = beta; CPE = enterotoxin; ETX = epsilon; ITX = iota; NetB = necrotic enteritis-like beta.

thick band of mostly neutrophilic infiltration. A variable number of gram-positive rods, usually non-sporulated, can be seen adhered to the necrotic intestinal mucosa and free in the lumen. Focal mucosal ulceration may also be present in the small intestine, colon, and cecum.

Diagnosis. The diagnostic criteria for *netF*-positive type A *C. perfringens* disease have not been defined. Demonstration of the *netF* gene in *C. perfringens* isolates recovered from the intestine of animals with characteristic clinical or pathologic changes is suggestive of the role of these isolates in disease, but not confirmatory. Currently, no tests for NetF toxin detection, such as ELISA, are available. Ruling out other causes of enteric disease in foals lends additional support to a diagnosis of NetF-associated disease.

Clostridium perfringens type C

Clinical presentation. Most cases of *C. perfringens* type C–associated disease are lethal. Affected animals have a history of acute or peracute onset of diarrhea, typically hemorrhagic, colic, and dehydration, followed rapidly by death. Occasionally, neurologic signs and sudden death have been reported.[42]

Etiology. C. perfringens type C strains produce 2 main exotoxins: alpha toxin (CPA) and beta toxin (CPB),[116] the latter being the main virulence factor responsible for inducing necrotizing enteritis, a fact that was demonstrated in several animal models for which both conventional and molecular Koch postulates have been fulfilled.[145]

Epidemiology. C. perfringens type C enterocolitis occurs most frequently in the form of sporadic cases or small clusters of cases.[146] The latter seems to recur yearly on the same properties. Because CPB is extremely trypsin-sensitive,[146] neonatal foals are the main age group affected by *C. perfringens* type C infections.[145] This predisposition is directly associated with the low level of intestinal trypsin activity during the first days of life, as a consequence of trypsin inhibitors in colostrum. A few cases of *C. perfringens* type C disease have been reported in adult horses, but the pathogenesis of such cases remains unknown. *C. perfringens* type C is not commonly isolated from feces or intestinal contents of normal foals. The reservoir of *C. perfringens* type C and conditions that allow it to overgrow and cause disease remain unknown, although it has been suggested that foals acquire it from the teats of their dams.[145]

Pathogenesis and immune response. The disease caused by *C. perfringens* type C starts with colonization and rapid proliferation in the intestine, and the production and secretion of CPB. Trypsin inhibitors in the colostrum, or other conditions that affects trypsin activity, prevent the lysis of CPB, which can cause enterocyte necrosis and hemorrhage.[145] In studies on piglets, however, it has been suggested that CPB crosses the epithelial barrier and diffuses into the lamina propria, inducing damage to endothelial cells. This activity would increase vascular permeability, leading to extravasation of fluid, plasma proteins, and erythrocytes, contributing to the necrotic effect on enterocytes.[108] The widespread thrombosis in the lamina propria seen in these cases is consistent with a vascular effect associated with this disease. This mechanism, however, has not been explored in horses. Given the acute and necrotizing nature of the disease, neutrophils are the main inflammatory cells that infiltrate the intestinal tissue.[108] However, the underlying mechanisms of the host immune and inflammatory response against *C. perfringens* type C in the intestine of horses have not been investigated. The complex nature of these events has been explored in pigs by analyzing the expression patterns of long non-coding RNAs and mRNAs involved in the piglet immune response against *C. perfringens* type C infection, in which at least 13 immune-related target mRNAs were identified to play potential roles in defense against bacterial infection in the intestine.[61]

Gross pathology. Gross lesions can occur in any section of the gut, including the small intestine, cecum, and colon. The distribution of lesions varies from segmental and well-demarcated areas to a more diffuse presentation. Intestinal contents are usually watery, brown-to-red, and foul-smelling. The intestinal mucosa is diffusely and severely hyperemic and/or hemorrhagic (Fig. 7), multifocally ulcerated, and may be covered by a pseudomembrane. Transmural edema, congestion, hemorrhage, and emphysema are common.[33] Lesions in other organs may include serous-to-serosanguineous pericardial effusion, pulmonary congestion and edema, and hemorrhages on the endocardial and serosal surfaces.[33,146]

Microscopic pathology. The distribution of microscopic lesions is consistent with that of gross lesions. Coagulative necrosis of the intestinal mucosa is a common feature, characterized by fusion and blunting of villi in the small intestine, collapse of crypts, loss of enterocytes, and

moderate-to-severe hemorrhage and congestion in all intestinal segments affected (Fig. 8). Mild-to-moderate numbers of neutrophils admixed with lymphocytes and plasma cells infiltrate the mucosa and submucosa. Fibrin thrombi are frequently seen in capillaries, small veins, and arterioles of the lamina propria, and occasionally in small-to-midsized arteries and veins of the submucosa.[33,146] In some cases, a diphtheritic pseudomembrane, composed of fibrin, cellular debris, and mixed bacteria, can be seen diffusely or multifocally attached to the necrotic mucosa. Numerous gram-positive rods are frequently observed multifocally in the lumen and/or attached to the denudated intestinal mucosa.[33,146]

Diagnosis. Isolation of *C. perfringens* type C from animals with necrotizing enteritis is highly suggestive of infection with this bacterium, given that it is rarely present in the intestine of healthy animals.[145,146] Genotyping of intestinal isolates should be performed, however, through a PCR assay.[116] Confirmation of *C. perfringens* type C-mediated disease requires the detection of CPB in intestinal contents and/or feces, in animals with the clinical, gross, and microscopic findings described above.[146] Toxin detection is almost universally achieved by ELISA. Freezing and/or adding trypsin inhibitors to samples of intestinal contents is helpful to maintain the lifespan of CPB for several weeks.[78]

Clostridioides difficile

Clinical presentation. The main clinical sign of *Clostridioides difficile*–associated disease (CDAD) is diarrhea, which is usually accompanied by one or more of the following: fever, colic, hyperemic mucous membranes, prolonged capillary refill time, tachypnea, tachycardia, dehydration, and abdominal distention.[35,36,146] The overall mortality rate in foals and adult horses with CDAD is variable between studies, ranging from 0 to 42%.[80,156] Significantly higher mortality has been reported in adult horses with CDAD compared with a non-CDAD group of horses.[97] In foals, the case fatality rate can be 0–42%; the case fatality rate is lower in older horses.[146]

Etiology. Clostridioides difficile is a gram-positive, anaerobic, spore-forming bacillus that is present in the soil and may be a normal inhabitant of the gastrointestinal tract of horses and other animals, including humans.[128] *C. difficile*–associated disease (CDAD) is mediated principally by 2 large toxins, toxin A (TcdA) and toxin B (TcdB).[73] Some strains of *C. difficile* may also produce a third toxin, ADP-ribosylating binary toxin (CDT), which may contribute to the pathogenesis of CDAD.[128] *C. difficile* causes diarrhea and enterocolitis in horses of all age groups.[35,36] CDAD has been reproduced successfully by administration of *C. difficile* vegetative cells and spores to pony foals.[4] In addition, *C. difficile* has been associated with the so-called duodenitis-proximal jejunitis syndrome, which was reproduced by administration of *C. difficile* toxins via gastroscopy.[5]

Epidemiology. C. difficile is a strict anaerobic bacterium that can survive in the environment in the form of highly resistant spores.[10] It is presumed that horses acquire spores from the environment, food, or water, and once in the gastrointestinal tract the spores germinate, proliferate, and release their toxins, which are responsible for the clinical signs and lesions of CDAD.

Environmental surveillance studies of *C. difficile* in veterinary hospitals are scarce; the reported prevalence is 4–8.3%.[153,155] The carrier rate for healthy foals is low, ranging between 0 and 3%.[10,155] However, clustering can occur with carrier rates as high as 29% in some farms.[10] The carrier rate of *C. difficile* in healthy adult horses is also low, ranging between 0 and 10%.[10,85,155] Adult horses and foals of all ages can develop *C. difficile* enteric disease.[65] Most CDAD cases are sporadic, with a few reported outbreaks.[38,80,105,157]

Risk factors for the development of CDAD in horses remain largely unknown; however, antibiotic use and hospitalization have been associated with the condition.[10,156] A retrospective study reported that a significantly higher proportion of horses with CDAD had been administered antimicrobials, had undergone surgery, or had been hospitalized, compared with those with non-CDAD.[97] Nevertheless, hospitalization is considered by some authors an unlikely risk factor in the epidemiology of this disease in horses.[156]

The gut microbiota of healthy and sick horses has been under intense scrutiny, but a precise link between dysbiosis and gastrointestinal disorders has not been determined. Antibiotics are associated with dysbiosis of the cecum and colon, allowing for *C. difficile* and other bacteria to grow.[11] A study showed that horses with colic were more likely than healthy horses to shed *C. difficile*, but no major microbiota differences were observed between groups.[120] Although macrolides, sulfonamides, β-lactam antibiotics, rifampicin, and gentamicin were traditionally associated with *C. difficile* diarrhea in horses,[55,97] all antibiotics are now considered to be a risk factor for development of CDAD. However, cases of CDAD can also occur in foals and adult horses without a history of antibiotic therapy.[12,156] Other proposed, although poorly documented, predisposing conditions for CDAD include stress, change of diet, transportation, starvation, nasogastric intubation, and surgical or medical treatment.[9,12] Coinfection and possible synergism of *C. difficile* and *C. perfringens* type C infection has been reported in foals.[143]

Pathogenesis and immune response. Once ingested, *C. difficile* spores germinate and the vegetative forms colonize the intestinal mucosa and produce the 2 major toxins, TcdA and TcdB. Horses may also be infected by direct ingestion of vegetative forms. A third route of endogenous proliferation of preexisting *C. difficile* spores in the intestinal lumen is likely to occur, but has not been proved in equids.[36,37] TcdA

and TcdB mediate the pathogenesis of CDAD by different mechanisms, which have been elucidated using animal models. These mechanisms are believed to be similar in horses, but they have not been confirmed to occur specifically in this species. Toxin binding to its receptor on the target cell, the intestinal mucosal epithelium, is a key event in the pathogenesis of CDAD. TcdA has several carbohydrate receptors (e.g., α-Gal epitope, Lewis X, Lewis Y, Lewis I) but a receptor for TcdB has not been identified. In vitro, TcdA is an enterotoxin, and TcdB is a cytotoxin but also an enterotoxin.[144] Both toxins are thus believed to contribute synergistically to damage in the mucosal epithelial cells by different mechanisms, including Ras GTPase inactivation with impairment of intracellular signaling pathways, cytoskeletal disruption by Rho glycosylation, and apoptosis. Cytokines and chemokines are released, which further intestinal lining damage by attracting neutrophils and other inflammatory cells.[128,144]

A third toxin, CDT, is also believed to contribute to damage of intestinal epithelium by cytoskeletal disruption and potentiation of the adherence of *C. difficile*.[128] CDT-producing strains are rare in horses, although they have been reported in Australia.[137] Serum from toxoid-immunized horses neutralize TcdA and TcdB in mice challenged with *C. difficile* spores.[158] However, it is still not known if antibodies produced after natural infection have the same effect.

Gross pathology. Gross lesions usually follow an age-related distribution throughout the gastrointestinal tract of horses, although not every case adheres to this rule.[35,37,70,146] Lesions in foals <1-mo-old are commonly located in the small intestine, with variable involvement of the colon and cecum, whereas the colon and cecum are most frequently affected in older animals.[35,36,70] The mucosal surface is usually dark-red and variably overlaid by a pseudomembrane in some cases[35,36,146] (Fig. 9). The intestinal contents are watery to pasty, green-to-brown, and occasionally hemorrhagic, the latter being especially common in younger foals. The serosal surfaces of the affected segments tend to be diffusely blue-to-red. The entire wall of the affected intestinal segment may be thickened and with a gelatinous appearance as a result of submucosal edema, especially in the colon and cecum. Lesions suggestive of endotoxic shock and/or septicemia, such as pulmonary congestion and edema, and serosal hemorrhages usually accompany the intestinal findings.[35]

Microscopic pathology. The microscopic lesions of CDAD are similar regardless of the area of the intestine that is affected, and are characterized by marked necrotizing-to-necrohemorrhagic enteritis, colitis, and/or typhlitis.[35,36,144] There are frequent areas of mucosal coagulative necrosis, which are covered by a pseudomembrane (Fig. 10). Mild-to-moderate numbers of neutrophils, lymphocytes, and plasma cells may infiltrate the lamina propria.[36] Focal areas of superficial mucosal ulceration from which fibrin and neutrophils are expelled to the lumen, the so-called volcano lesions, are occasionally seen, although they are not as common in horses as in other species.[35,36,144] Thrombosis usually occurs both in the mucosa and submucosa, and is often accompanied by marked congestion and/or hemorrhage.[35] Gram stain may highlight groups of short, gram-positive bacilli in the lumen, adhered to the mucosal surface, and/or within the necrotic lamina propria.[35] The submucosa may be markedly expanded by edema and dilated lymphatics, and transmural hemorrhage is reported in some cases.[35]

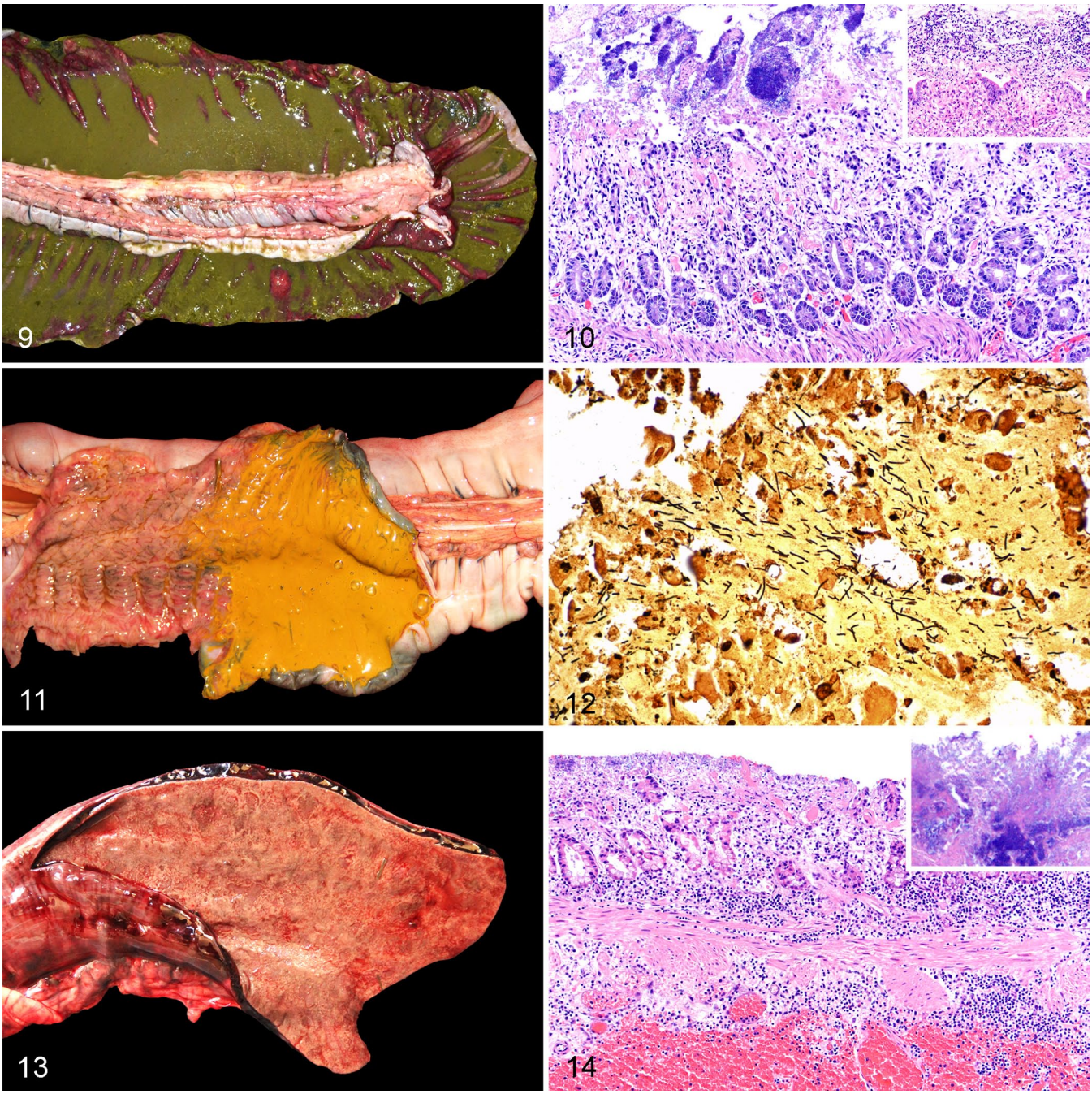
Diagnosis. Clinical signs and lesions are suggestive of CDAD, especially if there is a history of antibiotic administration and/or hospitalization, but these changes may be indistinguishable from those produced by other causes of enteric disease in horses.[146] Therefore, a presumptive diagnosis must be confirmed by detection of TcdA and/or TcdB in intestinal contents or feces.[35,36] Commercial ELISA kits can rapidly detect the toxins and are used routinely in diagnostic laboratories.[107] Toxin presence may also be demonstrated by cytotoxin assay in cell culture, which is more sensitive than ELISA, although this assay is time consuming and rarely used in routine diagnostic settings. *C. difficile* isolation is suggestive of CDAD, especially if associated with compatible clinical signs and lesions and isolation a toxinogenic strain.[10,69,157] Toxin typing of *C. difficile* isolates is, however, rarely performed in veterinary diagnostic laboratories.

Clostridium piliforme

Clinical presentation. Tyzzer disease (TD) is characterized primarily by severe hepatitis in young foals, affecting the intestinal tract in a reduced number of cases.[49] Foals are commonly found dead with no prior clinical signs being observed. When observed, reported signs include depression, anorexia, fever, tachypnea, tachycardia, jaundice, and lethargy, which progresses rapidly to shock, convulsions, and death. Severe diarrhea has been also reported, although not frequently.[132]

Etiology. Clostridium piliforme is a gram-negative, anerobic, filamentous, and obligate intracellular bacterium that causes TD, an enterohepatic syndrome that affects many animal species, including horses. No virulence factors have been characterized for *C. piliforme*, although a toxin is suspected to be produced by some or all strains of this microorganism.[93]

Epidemiology. TD occurs mostly in young foals, and it tends to recur annually on the same properties.[93] Cases of TD usually occur sporadically, but multiple cases had been reported in some farms. The source of *C. piliforme* and predisposing factors for foals to develop TD are unknown. Adult horses are resistant to disease, but they may carry *C. piliforme* in the



Figures 9–14. Equine enterocolitis of bacterial etiology. **Figure 9.** Colitis produced by *Clostridioides difficile*. The mucosa is diffusely hemorrhagic, and the content is liquid. **Figure 10.** Colitis produced by *C. difficile*. The mucosa is diffusely necrotic. Inset: higher magnification of volcano lesions. H&E. **Figure 11.** Colitis produced by *Clostridium piliforme* (Tyzzer disease). The mucosa is diffusely hemorrhagic, and the content is fluid. **Figure 12.** Colitis produced by *C. piliforme* (Tyzzer disease). Many filamentous silver-positive bacteria are in the cytoplasm of enterocytes and free in the intercellular spaces and lumen. Steiner. **Figure 13.** Necrotic enteritis produced by *P. sordellii*. There is severe and diffuse mucosal necrosis covered by a fibrous pseudomembrane and transmural hemorrhage. **Figure 14.** Necrotic enteritis produced by *P. sordellii*. There is severe and diffuse mucosal necrosis covered by a fibrous pseudomembrane, and transmural hemorrhage. Inset: higher magnification with clumps of intralesional rods. H&E.

gastrointestinal tract, where the bacteria proliferate and are passed through feces to young foals. *C. piliforme* endospores are resistant to heat and common disinfectants, and it is suspected that the source of *C. piliforme* for foals is feces from their dams. Coprophagy is common in foals <5-wk-old. Coincidentally, TD occurs between the second and fifth

week of age, and rarely after that.[135] In California, foals born between in March and April were 7.2 times more likely to develop *C. piliforme* infections than foals born at other times of the year. Similarly, 58% of the cases occurred in April and May on Kentucky horse farms.[45] In addition, foals from non-resident mares and foals born from mares <6-y-old were 3.4 and 2.9 times more likely to develop *C. piliforme* infection, respectively.[45]

Pathogenesis and immune response. The pathogenesis of TD in horses and other species is not completely understood. The common dogma is that *C. piliforme* spores are ingested from environments contaminated with the feces of carrier animals. The spores then colonize the intestine inducing direct damage to the enterocytes from where they are absorbed into the blood circulation, and reach other organs such as the liver and heart.[93] The interaction of *C. piliforme* with the host immune response has been studied mainly in mice. In these animals, serum IFNγ and IL-6 are elevated from day 1 to 14 after inoculation, and serum TNFα is elevated from day 1 to 28 after inoculation.[150] IL-12 levels were significantly higher in resistant than in susceptible mice, and histologic lesions in the liver were more severe if polyclonal antibodies against IL-12 and IL-6 were injected immediately prior to infection with the bacteria.[148,149] Depletion of either neutrophils or natural killer cells increased severity of disease in naturally resistant mice; however, macrophage depletion did not alter the course of infection in either resistant or susceptible strains of mice.[147]

Gross pathology. In most affected animal species, TD produces a triad of lesions involving the heart, liver, and intestinal tract.[47] This is, however, not a consistent feature in foals, which more frequently have only hepatic changes; colonic or cardiac lesions are only observed in a small percentage of cases.[49] The liver is frequently enlarged, with pale foci distributed randomly throughout the parenchyma. In addition, icterus and widespread serosal hemorrhages are consistent features of this disease.[93] When gross changes occur in the alimentary tract, they mainly affect the colon, which is diffusely reddened, containing liquid or semi-liquid contents (Fig. 11). Less frequently, pale foci or streaks are found in the myocardium.[49]

Microscopic pathology. The microscopic lesions in the liver are characteristic, and consist of random foci of coagulative necrosis, surrounded and infiltrated by large numbers of degenerate and viable neutrophils. Numerous filamentous bacilli, which are best visualized with silver stains or Giemsa, are seen in the cytoplasm of viable hepatocytes at the margin of these lesions.[49,93] When present, intestinal lesions are characterized by necrotizing colitis, submucosal edema, and infiltration of the lamina propria with lymphocytes, plasma cells, and neutrophils. The crypts are dilated and filled with necrotic debris. A few intracytoplasmic filamentous bacteria may be seen in the cytoplasm of intestinal epithelial cells and within necrotic debris (Fig. 12).[49,93]

Diagnosis. The characteristic macroscopic and microscopic changes, in association with the demonstration of intracellular filamentous bacteria in the liver, and less frequently in the colon, are significant diagnostically for TD in foals. *C. piliforme* cannot be cultured in conventional media, and propagation in embryonated eggs is needed, although this is not performed routinely in diagnostic laboratories.[146] PCR is available to detect the microorganism in tissues of affected animals.[49,93]

Paeniclostridium sordellii

Clinical presentation. In a series of 7 equine cases with *P. sordellii*–associated colitis, all of the animals had a history of acute colic.[99] There are no other reports of antemortem diagnosis of *P. sordellii*–associated enterocolitis in horses, and no other clinical information is available.[99]

Etiology. P. sordellii (previously known as *Clostridium sordellii*) is a gram-positive, sporulating, anaerobic rod, commonly associated with toxic shock syndrome in humans,[15,83,130] and gas gangrene in ruminants, pigs, and horses.[68] A potential role of this microorganism in gastrointestinal disease has been suggested in several animal species including lambs,[124] chickens,[115] and quail.[30] In addition, a case series of *P. sordellii*–associated enterocolitis has been reported in horses in California.[99] This bacterium produces several toxins, of which lethal toxin (TcsL) and hemorrhagic toxin (TcsH) are primarily responsible for its virulence.[106,152] The role of these or other toxins in equine enterocolitis, however, has not been elucidated.

Epidemiology. This microorganism can be found, albeit in small numbers, in the intestines of a small number of normal horses, and some as yet undetermined predisposing factors promote multiplication and likely toxin production. The cases described to date have been individual,[99] and no additional information is available about the epidemiology of this infection.

Pathogenesis and immune response. The association of *P. sordellii* with intestinal disease in horses is a relatively new finding, and neither conventional nor molecular Koch postulates have been fulfilled. Because this microorganism has been isolated from intestinal content of healthy horses, it is speculated that some animals may act as carriers of *P. sordellii*, shedding the microorganism in feces and infecting susceptible individuals via the fecal-oral route (authors' unpublished observations). In humans, leukocytosis is a remarkable clinical feature of *P. sordellii* infection; however, little is known about the underlying mechanism of this effect.[3] Putative virulence factors with predicted functions in

immune evasion have been detected in some *P. sordellii* strains, including an aureolysin that prevents complement activation during infection, the antimicrobial peptide LL-37, and a protease that cleaves human IgG.[29]

Gross pathology. P. sordellii–associated enterocolitis cases are characterized by lesions in either the small or large intestine, or both. There are segmental mucosal or transmural hemorrhages, with mucosal ulceration and diffuse pseudomembrane formation (Fig. 13). The intestinal wall is edematous, and the intestinal contents are dark-red and thin-to-pasty. The serosa of the affected intestinal segments is congested and/or hemorrhagic. Disseminated subendocardial, serosal, renal, and adrenal gland hemorrhages are usually present, suggestive of toxemia and/or disseminated intravascular coagulation.[99]

Microscopic pathology. Affected intestinal segments are characterized by mucosal and submucosal necrosis and leukocytic infiltrates, transmural hemorrhage, and edema (Fig. 14). Villus blunting and fusion is common in the small intestine. A pseudomembrane overlying the necrotic mucosa is composed of fibrin, desquamated enterocytes, red blood cells, leukocytes, and large numbers of gram-positive rods. Thrombi are present in mucosal and submucosal vessels.[99]

Diagnosis. Because *P. sordellii* has been found, although infrequently and in small numbers, in the intestine of a few healthy horses,[129] isolation of this microorganism without intestinal lesions has no diagnostic significance.[99] Given the gross and microscopic similarities of *P. sordellii*–associated enterocolitis with those produced by other agents, a presumptive etiologic diagnosis is only achieved by the detection of *P. sordellii* in association with intestinal lesions in affected horses by culture, IHC, and/or PCR assays, coupled with the exclusion of the most common causes of enterocolitis.[99]

Other clostridia

Other clostridia, including *Clostridium innocuum* and *C. sporogenes*, have been implicated as complications of CDAD in humans, and they are being investigated as potential etiologic agents of enterocolitis in horses (authors' unpublished observation). *C. innocuum* has been isolated from cases of recurrent diarrhea in humans with prior CDAD,[1] and it has also been associated with antibiotic-associated diarrhea characterized by diarrhea or severe colitis, including pseudomembranous colitis.[22] This microorganism has also been detected in more equine diarrhea samples than in feces of healthy controls (authors' unpublished observation).

Rhodococcus equi

Clinical presentation. Extrapulmonary manifestations of *Rhodococcus equi* infection in foals include, among others, pyogranulomatous or ulcerative enterotyphlocolitis, abdominal lymphadenitis, and intra-abdominal abscessation. Abdominal lesions occur in ~50% of cases of pneumonia.[144] Clinical signs of *R. equi* colitis include diarrhea, intermittent fever, diminished growth, and colic. Lymphadenitis and abdominal abscesses can be detected with imaging modalities, such as ultrasound and computed tomography.[122] Prognosis for survival is poor for foals with abdominal abscesses, although successful medical treatment has been reported.[121]

Etiology. R. equi is a facultative, intracellular, gram-positive coccobacillus that typically produces disease in foals 3-wk–5-mo-old.[64,74] Virulence of *R. equi* is attributed to expression of a plasma-encoded virulence-associated protein (VapA); this protein allows the organism to replicate within macrophages.[60] There are, however, avirulent strains, a fact that has to be taken into account during diagnostic procedures.[60,64]

Epidemiology. R. equi is a saprophytic soil organism in horse farms with endemic disease, and it is also found in the gastrointestinal tract of many herbivores. Fecal excretion of virulent *R. equi* is an important contributor to environmental contamination on equine breeding farms. Inhalation is the major route of infection in foals. The concentration of airborne *R. equi* increases in the environment of stables with warmer, drier, and windier weather conditions. The ingestion of sputum containing high concentrations of the bacterium is the likely source of the enteric form of the disease.[64]

Pathogenesis and immune response. As a soil-dwelling actinomycete, *R. equi* is often part of the normal intestinal flora of horses. Inoculation with this bacterium can occur as a result of inhalation, ingestion, and/or contamination of wounds or mucous membranes. Infection with *R. equi* typically induces a pyogranulomatous inflammatory response, and the bacterium is taken up by macrophages.[60] Virulent strains of *R. equi* have a plasmid (pVAP) that allows the bacterium to survive and replicate within macrophages.[151] These strains are able to modify the phagocytic vacuole of host macrophages to prevent acidification and fusion with lysosomes. However, other leukocytes (e.g., neutrophils) are effective in phagocytizing and killing *R. equi*. Uptake of *R. equi* by macrophages is facilitated by complement (specifically Mac-1, a type 3 complement receptor) or may occur via a mannose receptor.[151]

Gross pathology. Respiratory disease is the most common manifestation of *R. equi* infection in foals. However, extrapulmonary manifestations of *R. equi* infections are also reported in ~50% of cases. Intestinal disease associated with *R. equi* equi infections can affect either the small or large intestine; however, the most severe changes are typically noted in the cecum, large colon, and regional lymph nodes.[146] Grossly, enteric *R. equi* infections are manifest as mucosal and/or submucosal nodular thickenings, with occasional

crateriform ulcers (Fig. 15). Regional lymph nodes are often firm and enlarged with grossly apparent caseous exudate on cut section[146] (Fig. 16).

Microscopic pathology. Histologically, the mucosa and submucosa are expanded by pyogranulomas with variable numbers of intrahistiocytic and free gram-positive coccobacilli[100,146] (Fig.17). A similar pyogranulomatous infiltrate is noted within the regional lymph nodes.[100]

Diagnosis. Antemortem diagnosis may be challenging, and it is based on clinical signs, coupled with imaging and detection of virulent strains of *R. equi* in feces. In dead horses, gross and histologic findings are often highly suggestive of *R. equi* infection. However, definitive diagnosis requires the detection of virulent strains of *R. equi* in intestinal contents or feces by culture and/or PCR assay. Isolates should be tested for the virulence plasmid, given that avirulent strains do exist.[60,64]

Potomac horse fever

Clinical presentation. Potomac horse fever (PHF; also known as equine monocytic ehrlichiosis, equine ehrlichial colitis, and equine neorickettsiosis) is a rickettsial disease that affects adult horses and foals. The most common clinical presentation of PHF is diarrhea and fever, which when combined, represent 93% of the cases. Neutropenia is the most common early hematologic abnormality, although marked leukocytosis can occur within a few days of onset of the disease. Serum biochemistry abnormalities, including hyponatremia, hypochloremia, and hypoalbuminemia, are observed commonly in diarrheic horses with PHF. Laminitis is a common complication of PHF, and can occur in up to 40% of affected horses.[123] Abortion as a result of fetal infection with *N. risticii* has been reported to occur at 90–250 d of gestation, after experimental or natural infection with this microorganism.[123]

Etiology. Neorickettsia risticii (formerly *Ehrlichia risticii*) is the causative agent of PHF. However, recently a new *Neorickettsia* sp. named *N. findlayensis* was isolated from horses with PHF.[136] *Neorickettsia* spp. are intracellular, gram-negative, endosymbiont bacteria of digenean trematodes that parasitize aquatic insects and snails. Horses become infected when they ingest some of the latter or the trematode directly, and the bacteria invade their leukocytes.[114] PHF has been reproduced experimentally by different means, including oral ingestion of infected trematodes and aquatic insects, intravenous inoculation of infected monocytes, and subcutaneous or intravenous inoculation of the bacteria.[40,59,81,109]

Epidemiology. PHF is a seasonal disease in North and South America. In the United States, the disease has been confirmed serologically in 41 states. In Canada, clinical cases have been confirmed in 5 provinces. Clinical disease in horses was reported along the border between Brazil and Uruguay, and *N. risticii* has been detected by molecular methods in horses from Brazil and in bats from Brazil and Argentina.[23,24]

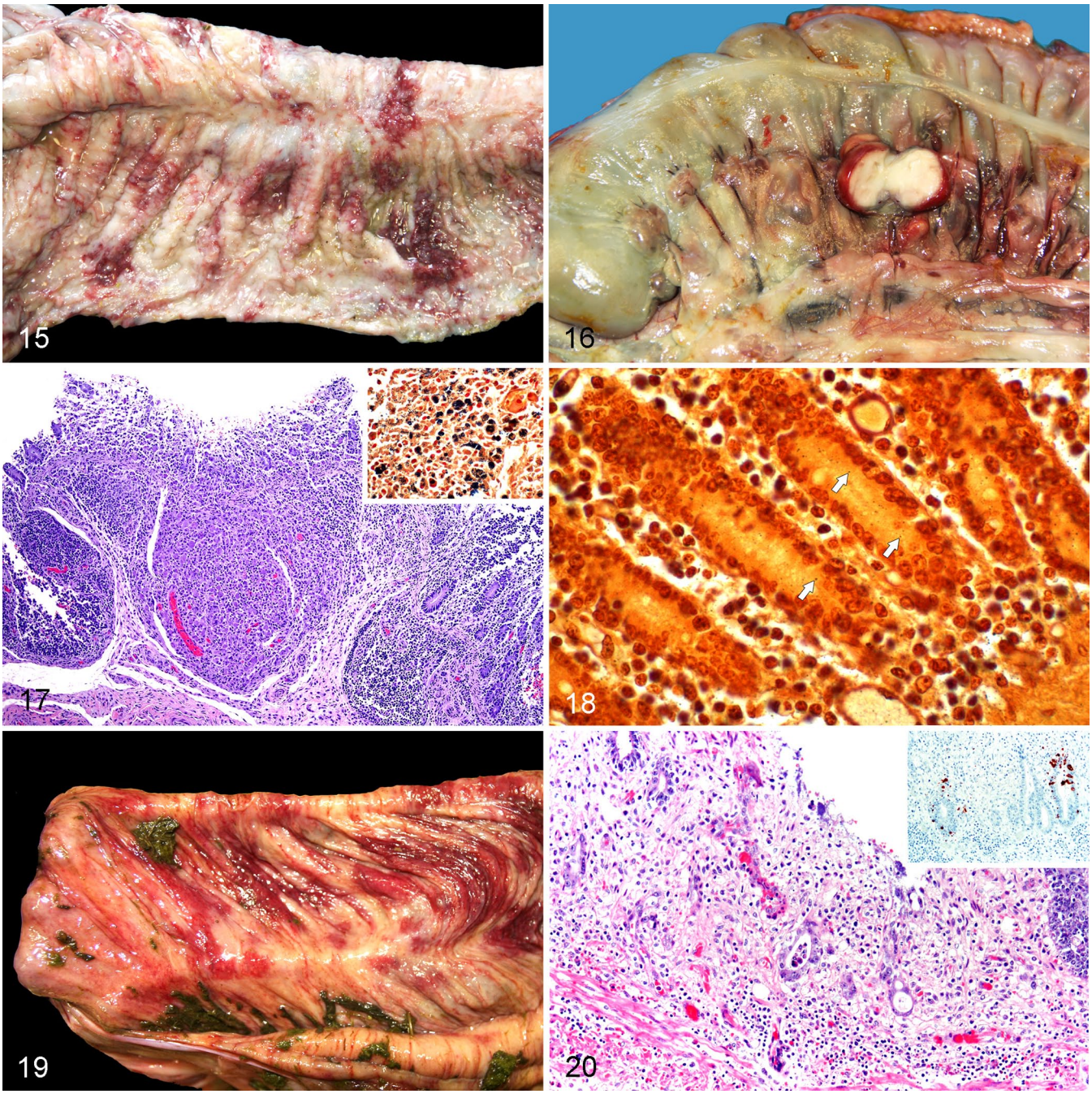
PHF cases typically occur in spring and summer in North America, with a peak incidence in July and August. However, cases may occur as late as November. In Brazil, the peak incidence of the disease is in January and February. The number of cases can vary greatly from year to year in a particular region.

Neorickettsia spp. are obligatory endosymbionts of trematode hosts. The trematode has a complex life cycle involving miracidia and sporocysts in its snail host, free-swimming cercariae, and metacercariae in aquatic insects (i.e., caddisflies and mayflies). The adults lay eggs in the intestine of insectivorous bats. The bacterial survival, distribution, and population density are likely dependent on the host trematode population, and in turn, the survival and population density of the trematode are dependent on its 2 intermediate and definitive hosts. The host(s) of the recently described *N. findlayensis* and the life cycle of its encysted trematode remain unknown. Once inside the host, *Neorickettsia* spp. replicate in monocytes, macrophages, mast cells, and intestinal epithelial cells.

Both *N. risticii* and *N. findlayensis* cause disease in experimentally infected horses, and the causative agents have been recovered from blood and feces of infected horses.[136]

Pathogenesis and immune response. Neorickettsia spp. invade equine leukocytes, mainly circulating monocytes and tissue macrophages, mast cells, and also intestinal crypt epithelial cells.[114] Replication occurs in the phagosome, and the organism is transported to other organs via infected blood monocytes. The mechanisms behind disruption of the enteric mucosa and subsequent diarrhea are not completely understood, but a combination of direct epithelial cell damage and the effects of infiltrating infected macrophages and mast cells on the lamina propria is believed to play a role.[114] Diarrhea may also occur as a result of impaired sodium chloride absorption.[118] The 51-kDa antigen (P51) is an outer membrane protein that plays an important role in the recognition and host immune response against *Neorickettsia* spp.[51]

Gross pathology. Lesions in the gastrointestinal tract are most severe in the cecum and large colon.[144] Contents are usually watery, malodorous, and brown. Areas of hyperemia, petechial hemorrhages, and pinpoint ulcers in the large colon and cecum are usually described.[28,39] Associated cecal and colonic lymph nodes are enlarged. Similar changes, albeit less consistent and severe, may be detected in the small intestine. Changes in the small colon are uncommon. Additionally, gastric ulceration, oral vesicles, and laminitis are seen



Figures 15–20. Equine enterocolitis of bacterial or viral etiology. **Figure 15.** Hemorrhagic and ulcerative mucosa in colitis caused by *Rhodococcus equi.* **Figure 16.** Colonic mesenteric lymphadenitis caused by *R. equi.* The lymph node is enlarged and effaced by white, soft exudate. **Figure 17.** Submucosal granulomas in colitis caused by *R. equi.* H&E. Inset: higher magnification of the submucosal granulomas with large numbers of gram-positive coccobacilli in the cytoplasm of macrophages. Gram. **Figure 18.** Lymphoplasmacytic infiltration of the lamina propria in colitis caused by *Neorickettsia risticii*. Within macrophages and enterocytes are pinpoint, silver-positive, ~1-µm organisms compatible with *N. risticii* (arrows). Steiner. **Figure 19.** Diffusely hemorrhagic mucosa in enteritis caused by equine coronavirus. Photo courtesy of Dr. Federico Giannitti. **Figure 20.** Mucosal necrosis with crypt dilation in enteritis caused by equine coronavirus. H&E. Inset: immunohistochemistry for equine coronavirus. Photo courtesy of Dr. Federico Giannitti.

occasionally in some cases.[28,144] Other lesions such as pulmonary congestion and edema can occur, and are believed to be secondary to endotoxemia.[144]

Microscopic pathology. Histologic changes occur mostly in the large, and to a lesser extent, the small intestine.[144] There is loss and necrosis of the superficial epithelium, and

the crypts may be necrotic and/or filled with inflammatory cells and debris.[7,28,39] An intense infiltrate of mixed inflammatory cells, with predominance of lymphoid cells in the lamina propria that reaches the submucosa, is encountered commonly.[28] Strands of fibrin, debris, and degenerate inflammatory cells may also be observed in the lamina propria and/or overlie the mucosa. Severe congestion and mucosal hemorrhages are frequently seen in the hyperemic areas detected grossly.[28,39] Silver stains highlight aggregates of <1-μm diameter organisms within glandular epithelial cells and macrophages in the lamina propria[133] (Fig. 18). By electron microscopy, bacteria are detected either as elementary bodies or as morulae within the cytoplasm of epithelial cells, macrophages, and mast cells of the large colon principally.[28,114,144]

Diagnosis. Clinical signs and gross and histologic findings may be suggestive of PHF, especially if compatible organisms are detected by silver stains in histologic sections.[146] However, other agents such as *Salmonella* spp. and *Clostridium* spp. could have similar clinicopathologic presentations, and silver stains may be difficult to interpret in some cases (authors' unpublished observations). Therefore, appropriate ancillary tests to rule out other causes of enterocolitis are warranted. PHF diagnosis is confirmed by PCR assay of feces, intestinal contents, and peripheral blood buffy coat or whole blood.[13,14] Serology by indirect immunofluorescence antibody assay may be useful for antemortem diagnosis, although the kinetics of antibody production are very variable and influenced by vaccination.[7,84,104] Nevertheless, a 4-fold titer increase in 3–4 wk is relevant diagnostically.[7]

Lawsonia intracellularis

Clinical presentation. Lawsonia intracellularis is the causative agent of equine proliferative enteropathy (EPE). Most EPE cases occur during the fall and winter months in North America. The most common clinical complaints include lethargy, anorexia, fever (>38.5°C), peripheral edema (ventral abdomen and distal limbs), and weight loss. Colic and diarrhea are also reported, and the diarrhea can vary from cowpie to watery; however, many affected foals have normal fecal consistency.

Hypoproteinemia as a result of hypoalbuminemia is the most consistent clinicopathologic finding, including in foals without diarrhea. The pathogenesis of the hypoalbuminemia is unknown but may result from decreased feed intake, malabsorption, and protein-losing enteropathy. Other nonspecific hematologic and serum biochemistry abnormalities include anemia or hemoconcentration, leukocytosis or neutropenia, and increased inflammatory markers (e.g., fibrinogen, SAA) and muscle enzyme activity. Electrolyte abnormalities include hypocalcemia, hypochloremia, and hyponatremia.[46,75]

Epidemiology. The epidemiology of EPE in horses is poorly understood. This disease affects foals <1-y-old, although cases have been reported in older horses. Transmission is likely via ingestion of fecal material from infected animals. EPE affects weanling and yearling horses typically. There seems to be a seasonal pattern to the occurrence of this disease, which in North America often occurs between August and January. Cases in other times of the year have, however, been reported.[103]

Pathogenesis and immune response. L. intracellularis infection occurs via the fecal-oral route. Once ingested, the microorganism enters enterocytes within 3 h of infection. The hallmark of this disease, intestinal mucosal hyperplasia, is the result of rapid and unchecked division of crypt epithelial cells by an as yet unknown mechanism.[103] *L. intracellularis* has type III secretion system components; in other enteric pathogens, these components contribute to alteration of apoptosis, cellular invasion, and immune suppression.[2]

Gross pathology. The main gross changes of EPE occur in the distal small intestine, where the mucosa is rugose and may have a polypoid appearance.[103,144] These lesions are best seen on the lumen side, but can also be apparent from the serosa.

Microscopic pathology. Crypt epithelial hyperplasia occurs with elongation and branching of crypts and villi, and reduced numbers of goblet cells. Inflammatory cell infiltration is notoriously absent. Silver stains reveal the presence of the microorganism in the apical portion of enterocyte cytoplasm.[77,103]

Diagnosis. The clinical diagnosis of EPE is based on detection of hypoproteinemia characterized by hypoalbuminemia, coupled with serology and/or PCR assay results. The gross and microscopic postmortem findings are suggestive of the disease. Confirmation should be based on detection of the microorganism by PCR assay and/or immunohistochemistry.[103]

Other bacterial agents of colitis in horses

Other bacterial pathogens, including *Actinobacillus equuli*,[34] *Streptococcus equi*,[34] *Listeria monocytogenes*,[94,154] and *Klebsiella pneumoniae*,[146] have been associated rarely with colitis in horses. However, definitive evidence of their role in alimentary disease of horses is lacking, and confirmation of their role is difficult because most of these agents can be found in the intestinal content of clinically healthy horses. Enterocolitis associated with bacteria of the *Mycobacterium avium* complex have been reported, albeit rarely, in horses. The gross lesions in these horses were similar to those described in cases of Johne's disease in ruminants, and consisted of granulomatous colitis, lymphangitis, and lymphadenitis with myriad intralesional acid-fast bacteria.[18,25]

Escherichia coli is considered a cause of diarrhea in neonatal foals, although enterocolitis has not been associated with this microorganism.

Antimicrobial therapy for bacterial diseases

The use of antimicrobial therapy in horses with bacterial typhlocolitis remains controversial and is generally not recommended. The use of broad-spectrum systemic antimicrobial drugs, usually a combination between a beta-lactam and an aminoglycoside, is generally only considered for diarrheic horses with 2 episodes of fever 12 h apart in a neutropenic patient (absolute neutrophil count $<0.5–1.0 \times 10^9$/L [500–1,000/μL]). Oxytetracycline is the antimicrobial treatment of choice for horses with neorickettsiosis. A combination of oral macrolides and rifampin, or single therapy with systemic tetracyclines, is recommended for the treatment of foals with *L. intracellularis* infection. Horses generally improve in 24–48 h after treatment is initiated, although relapse of clinical signs or poor response to treatment has been reported anecdotally.[123]

Viral diseases

Equine rotavirus

Clinical presentation. Disease caused by equine rotavirus can be seen in foals 2–160-d-old, with most cases occurring at 5–35-d-old.[6] The severity of the diarrhea depends on the degree of maturation of the gastrointestinal tract. Thus, infection with rotavirus in foals <2-wk-old is associated with life-threatening diarrhea, whereas diarrhea is minimal in older foals.[41,142]

Etiology. Rotaviruses belong to the family *Reoviridae*, and are non-enveloped, double-stranded RNA viruses with a diameter of ~80 nm.[82] Rotaviruses are classified into 7 antigenically distinct groups (A–G) based on variations in a common inner capsid protein (VP6).[27,90] Equine rotavirus group A (ERVA) can be further classified based on the characteristics of VP7 and VP4 proteins into G (glycoprotein) types or P (protease sensitive) types, respectively. G3P[12] and G14P[12] are among the most prevalent serotypes isolated from horses in several countries.[82] ERVAs are a frequent cause of diarrhea in foals.[82]

A novel equine rotavirus group B (ERVB) has been identified in Kentucky in foals with diarrhea and with no other enteric pathogens detected. This virus had 96% identity with group B rotaviruses previously found in ruminants, suggesting that the ERVB that affected these foals originated from ruminants.[142]

Epidemiology. Equine rotaviruses are ubiquitous in horse populations, with a high seroprevalence in adult individuals. Equine rotavirus infections occur commonly in foals <3-mo-old, with a prevalence of 20–77% in diarrheic foals.[6,48,63,82,125] Transmission is via the fecal-oral route through contamination of fomites by feces.[6,113] This virus is highly contagious with high morbidity rates, but mortality rates are low.[6,82] Co-detection with bacteria (e.g., *Salmonella* spp.), other viruses (e.g., equine coronavirus), and parasites (e.g., *Cryptosporidium* spp.) is reported frequently, but the clinical significance of these coinfections is yet to be determined.[16,125] The frequent occurrence of equine rotaviral infection cases during the winter and spring may be attributed to high numbers of susceptible animals during this time (foals <3-mo-old).[27,92,98]

In the cases of diarrhea associated with ERVB recently described, foals developed diarrhea when ~48-h-old, and diarrhea lasted for 3–4 d. Morbidity in affected farms was up to 100%. Lethality was very low in foals that received intensive medical treatment.[142]

Pathogenesis and immune response. Few experimental infection studies with rotavirus have been performed in foals, and the pathogenesis of rotaviral infections in this species has been extrapolated to some degree from other species.[6] In general, rotaviruses produce diarrhea through the destruction of terminally differentiated enterocytes covering the tips of the intestinal villi.[144] Young animals are particularly susceptible to rotaviral infections given the high proportion of terminally differentiated enterocytes and slow epithelial cell turnover. Virions are released by lysis of infected cells, and there is subsequent blunting of intestinal villi. The rotaviral genome encodes several virion proteins (e.g., VP1–4, 6, 7) and nonstructural proteins (e.g., NSP1–6). NSP4 is thought to be an enterotoxin that probably contributes to intestinal lesions by impairing sodium and/or glucose transport, reducing disaccharidase activity, and disrupting calcium homeostasis.[134] The pathogenesis of the recently described ERVB-associated cases of diarrhea in foals has not been determined, but it is likely similar to the pathogenesis of ERVA diarrhea.

Gross pathology. Grossly, affected foals often have liquid intestinal content.[146] Information on gross and microscopic pathology of rotavirus-infected foals is scant, because the disease is rarely fatal and autopsy is infrequently performed on these foals (authors' unpublished observations). In some experimentally infected foals, there was reddening and edema of the small intestinal, and occasionally large intestinal, mucosa.[62] No information is available on the gross pathology of foals with ERVB-associated diarrhea.

Microscopic pathology. As stated above, information on gross and microscopic lesions of rotavirus-infected foals is scant. Rotaviruses infect absorptive epithelial cells at the tips of the villi in the small intestine, and there is typically sloughing or exfoliation of the infected enterocytes with blunting and atrophy of the affected villi. Others report small intestinal

changes include edema, a mononuclear inflammatory infiltrate, and vacuolation of the cytoplasm of infected enterocytes.[6] Histologic findings in the large intestine are generally mild and nonspecific. No information is available on the gross pathology of foals with ERVB-associated diarrhea.

Diagnosis. Clinical, gross, and microscopic changes are nondiagnostic, although they can be interpreted as suggestive of rotaviral infection. Several assays can be used in the detection of rotaviral infection, including electron microscopy, latex agglutination, ELISA, reverse-transcription PCR, immunogold-based horizontal flow membrane assay (ImmunoCard STAT!; Meridian Bioscience), and immunochromatography (Dipstick "Eiken" Rota dipstick; SA Scientific).[82] These tests can be performed on intestinal content and/or feces. ERVB infection in foals was diagnosed by Illumina-based metagenomic sequencing.[142]

Equine coronavirus

Clinical presentation. Horses develop clinical signs compatible with equine coronavirus (ECoV) infection 48–72 h after infection,[43,95] but clinical signs resolve promptly (days to 1 wk) with minimal supportive care[112]; fecal shedding occurs for 3–25 d.[43,110,112] The most common clinical signs described in horses with ECoV infection are inappetence, lethargy, and fever. Changes in fecal consistency (from soft feces to watery diarrhea) and colic are reported in ~20% of affected horses.[101,102] Neurologic signs, including head pressing, ataxia, circling, nystagmus, proprioceptive deficits, recumbency, and seizures, have been reported in 3% of ECoV cases.[43,110] The development of neurologic signs has been associated with hyperammonemic encephalopathy, which is thought to be secondary to increased ammonia production within or absorption through the affected gastrointestinal tract.[50,112] However, this remains speculative and definitive proof is lacking.

Etiology. ECoV causes fever, gastrointestinal disease, and neurologic disease in adult horses and foals.[32,110] Coronaviruses are enveloped, single stranded, RNA viruses, of 100–130-nm diameter; they are divided into 3 genera: *Alphacoronavirus*, *Betacoronavirus*, and *Gammacoronavirus*.[144] ECoV belongs to the genus *Betacoronavirus* along with other animal and human coronaviruses.[57,111] Cases of ECoV-associated disease have been reported in Japan, the United States, and Europe.[17,56,91,102] ECoV Japanese sequences are closely related to NC99, the isolate obtained in the United States.[96,160] Clinical signs in adult horses have been reproduced experimentally using ECoV-containing fecal material.[95,119]

Epidemiology. Infections with ECoV occur more commonly in adult horses (>5-y-old) and the prevalence is 20–55%, depending of the age of the horses.[43,91,101,102,110] However, controversial results have been reported regarding the role of ECoV on diarrhea in foals. ECoV was found only as a coinfection with other etiologic agents in diarrheic foals in Kentucky,[125] whereas a Japanese study failed to identify ECoV in fecal samples from diarrheic foals.[96] ECoV-associated outbreaks in adult horses have been reported in riding, racing, and show animals, and less commonly in breeding farms.[112] The seroprevalence of ECoV in U.S. horses had a higher odds ratio (OR = 1.9) for draft horses compared to other breeds. Similarly, in Japan, draft horses have a higher infection rate.[72]

Cases of ECoV infection are most commonly reported during the colder months of the year.[112] Transmission appears to be via the fecal-oral route.[95] Seventy-five per cent of the horses that develop clinical signs consistent with ECoV infection test positive in feces,[112] and, in some horses, ECoV can be detected in nasal secretions. However, it is currently unknown whether the detection of ECoV in nasal samples indicates fecal contamination or ECoV nasal replication and shedding.[112] The morbidity of ECoV is 10–83%,[43,101,102,110] but mortality is uncommon.

Pathogenesis and immune response. Similar to other coronaviruses, ECoV uses the viral S protein to attach to a receptor in enterocytes, but the cellular receptor is not known.[57] After binding to the receptor, the viral fusion protein promotes merging of the viral and enterocyte membranes, with release of viral nucleocapsids to the cytoplasm, followed by hijacking of the host cell protein production system to form viral proteins. This process has been proven to be highly cytocidal in enteric coronaviral infections of other domestic animals, such as swine,[71] although it has not been studied specifically in the case of ECoV. Cycles of enterocyte lysis, with villus atrophy, infiltration of mononuclear inflammatory cells as a defense response, and enterocyte hyperplasia as a later compensatory event, are believed to be part of the host-pathogen interaction cycle.[159] In horses, ECoV seems to induce a higher degree of necrosis in the crypt epithelial cells, similar to the lesions caused by bovine coronavirus, but different from cases of alpha coronavirus infection in pigs.[50]

Gross pathology. Reports of gross pathology are limited.[32,50,57] Descriptions include reddening of the mucosa of the small intestine and colon, with pseudomembrane formation in the small intestine[50] (Fig. 19). Intestinal contents may be watery and blood-tinged.[50] Submucosal and mucosal edema in the small and large intestine, respectively, has been described.[32]

Microscopic pathology. Histologically, there is severe necrotizing enteritis, affecting mainly the jejunum and ileum[50] (Fig. 20). Affected segments have a combination of diffuse villus attenuation, necrosis of the surface and crypt epithelium, presence of a pseudomembrane, and hemorrhage and thrombosis of the lamina propria. Intracytoplasmic, eosinophilic

inclusion bodies, generally within clear vacuoles, are detected occasionally in crypt enterocytes. Hemorrhage may also be observed in the lamina propria of the large colon, without necrosis or inflammation.

Extraintestinal lesions include Alzheimer type II astrocytosis in the cerebral cortex of animals with secondary hyperammonemic encephalopathy, and toxemia-associated lesions (i.e., hemorrhages in adrenal glands and thymus, pulmonary congestion and edema).[50]

Diagnosis. Compatible clinical signs and postmortem changes should prompt further investigations into the role of ECoV, especially in cases that have tested negative for other agents associated with enteric disease.[146] Diagnosis is confirmed by PCR assay of fecal material or intestinal contents. Immunohistochemistry, direct electron microscopy, fluorescent antibody test, and antigen ELISA are confirmatory tests.[32,50,111]

General prevention and control of bacterial and viral enterocolitis in horses

Horses with enterocolitis usually pass frequent and voluminous amounts of feces, and therefore they contaminate the environment and can act as a source of infection for other horses. Therefore, universal and basic concepts of disease prevention, control, and biosecurity should always be considered and implemented whenever possible. Reducing the environmental contamination by thorough cleaning and disinfection is paramount. Diarrheic horses should be housed in an isolation unit and handled with barrier precautions. Feces, bedding, and any items in the stall should be considered contaminated, and be cleaned and disinfected prior to reuse.

Similar to humans, certain antimicrobials have been associated with the development of diarrhea in horses and therefore restrictive use is absolutely necessary, particularly in high-risk patients. Additionally, some enteropathogens can be contagious. *Salmonella* spp., for example, can be transmitted to other horses and remain in the environment for prolonged periods despite rigorous cleaning and disinfection. This pathogen also poses a risk to humans, and is a major zoonotic risk.

Various factors such as stress, transportation, hospitalization, altered motility (ileus, colic, impactions), general anesthesia, and fasting can contribute to the development of diarrhea. These stressors likely predispose to changes in the intestinal microbiota, favoring growth of potential enteropathogens.

Biosecurity and biocontainment approaches to minimize oral exposure to *Salmonella* spp. to susceptible hosts can be completed without markedly laborious or expensive practices. Avoiding overcrowding and overgrazing can reduce the load of *Salmonella* spp. in the environment.[58] Cleaning and disinfecting equipment and areas contaminated with feces prevents oral exposure and spreading of contaminated material. Horses that tested positive for *Salmonella* spp. should be isolated from other horses for 30 d before the test is repeated. During this period, all pieces of equipment that were in contact with the infected horses should be disinfected and kept away from contact with other horses. Bleach is an effective disinfectant against *Salmonella* spp. Footbaths at the entrance of the stalls help preventing mechanical spread of contaminated material, but they are only effective if they are kept clean and free of organic material. Manure should be disposed in a way that no horses will have contact with it. Historically, it has been recommended that after 30 d of isolation, *Salmonella* spp. cultures should be repeated on 5 fecal samples taken at 12-h intervals and, only after a horse tests negative on these 5 samples can the horse be allowed to commingle with other horses.[58]

Cleaning and disinfection remain the main strategy to reduce bacterial contamination of the environment, which may in turn reduce the occurrence of *C. perfringens*–associated diarrhea. The effective elimination of *C. perfringens* type A is not possible because this organism is ubiquitous in the environment. *C. perfringens* has been isolated from mare's teats and body, but it is unknown whether instituting hygiene protocols for the mare could prevent or decrease the risk of disease development.[138] An autogenous vaccine for the prevention of type A *C. perfringens* enterocolitis in foals has been reported, but data on its efficacy are not available.[139]

It is speculated that increased availability of nutrients may promote *C. piliforme* overgrowth in the gut of foals and their dams, as occur with other peracute clostridial diseases in foals. Therefore, intuitively avoiding sudden changes in the diet of mares may be helpful in controlling the disease in foals. This may prove difficult to manage however, particularly in seasons such as the spring when rainfall dramatically affects the growth of grasses, and a marked increase in the protein and non-protein nitrogenous components of the forages. For most enteropathogens in horses, there are no commercially available vaccines; an exception is a vaccine for PHF, but its efficacy is unknown.

Concluding remarks

In ~50% of the cases of enterocolitis in horses, the etiology remains undetermined despite the observation of clear clinical signs and/or postmortem findings.[79] The etiologic diagnosis of equine enterocolitis relies on gross and microscopic lesions coupled with ancillary tests.[144] Gross and microscopic lesions of the alimentary tract of horses with enterocolitis may be very similar, regardless of the etiology involved, which is a complicating factor in determining the etiology.[89] In addition, some of the bacterial agents associated with enterocolitis in horses are found in the intestine of normal animals, which can present a diagnostic challenge. An example of progress is the discovery of NetF, a toxin produced by some strains of *C. perfringens* type A.[88] Although the definitive role of this toxin in cases of enterocolitis has

not been established definitively, it has been postulated that strains of *C. perfringens* type A that produce NetF may be responsible for the condition. If this proves to be true, isolation of *C. perfringens* from the intestine of horses should be followed by PCR typing, including a search for the *netF* gene.

Acknowledgments

We thank the California Animal Health and Food Safety Laboratory for its support.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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