Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

Lawsonia intracellularis

34

Nicola Pusterla, Connie J. Gebhart, Jean-Pierre Lavoie,* and Richard Drolet*

Etiology

Lawsonia intracellularis is the etiologic agent of the recently recognized and emerging intestinal disease in horses called *equine proliferative enteropathy* (EPE). *Lawsonia intracellularis* is an obligate intracellular, curved, gram-negative bacterium that resides freely within the apical cytoplasm of infected intestinal enterocytes.[1] It causes proliferation of the affected enterocytes, resulting in a thickened small and sometimes large intestine. *Lawsonia intracellularis* can only be grown in vitro in cell culture and requires a specific atmosphere for growth. Besides horses, *L. intracellularis* infects many species of domestic and wild animals, including pigs, hamsters, rabbits, foxes, deer, ferrets, ostriches, and nonhuman primates. Equine proliferative enteropathy was first reported in horses in 1982 by Duhamel and Wheeldon.[2] Since 1996, several reports of sporadic cases and outbreaks on breeding farms have been described.[3-20] In the last few years, reported cases of EPE have been increasing, occurring primarily in postweaning foals and occasionally in adult horses. The disease has almost reached a worldwide occurrence and has been reported in the United States, Canada, Europe, South Africa, Australia, Brazil, and Japan.

Molecular investigations of *L. intracellularis* isolates from proliferative enteropathy lesions of a variety of animal species, including horses and hamsters, showed 98% homology of the 16S-ribosomal deoxyribonucleic acid (rDNA) gene to pig isolates.[21] Moreover, phenotypic characterization of outer membrane proteins and immunoblots of different *L. intracellularis* isolates using several antibodies and more sensitive molecular characterizations of the *L. intracellularis* genome demonstrated only minor differences among isolates. None of these differences appears to be antigenically relevant. Recently, the whole genome of a porcine *L. intracellularis* isolate was sequenced and analyzed for the presence of variable number tandem repeat (VNTR) sequences.[22] Variable number tandem repeat sequences in the genomes of prokaryotes are often associated with a high level of polymorphism and enable bacterial strain differentiation with substantial discriminatory power. Use of these *L. intracellularis* VNTR sequences provides a sensitive method for analysis of the genetic relatedness of *L. intracellularis* bacteria or DNA obtained from various temporal and geographic locations and from various animal species. This provided insight into the phylogenetic relatedness of these isolates. Molecular VNTR sequence profiles of *L. intracellularis* isolates from various documented outbreaks of proliferative enteropathy occurring in pigs, horses, ostriches, spider monkeys, ferrets, and hamsters were analyzed. The patterns that emerged provide some insight into the sources and phylogenetic relatedness of *L. intracellularis* isolates from different species. Variable number tandem repeat sequence types obtained from pigs were very different from those obtained from horses or other nonpig species. Little or no genetic variation was found between isolates from within outbreaks for any animal species or in multiple temporal samples taken from the same outbreak site. Slight variations between isolates obtained from outbreaks at different geographic locations were found, but these differences were minor. Marked variation in VNTR types were found, however, between isolates from pig sources and those obtained from non-pig sources including horses (Fig. 34-1).

*The authors acknowledge and appreciate the original contributions of these authors, whose work has been incorporated into this chapter.

Epidemiology

In pig populations, intestinal adenomatosis is maintained by chronic carriers, allowing transmission of *L. intracellularis* from one pig generation to the next.[23] Mice and rats are important reservoirs of *L. intracellularis* on piggeries with the percentage of polymerase chain reaction (PCR)-positive animals varying substantially between farms (4% to 83%).[24-26] Rodents appear to be suitable reservoir hosts because of their susceptibility to *L. intracellularis*, their close contact to domestic animals, and their high reproductive rate, which maintains *L. intracellularis* across generations. The source of infection has not been determined for horses. Exposure to pig feces has been suggested as a potential source of infection for horses since the first reported cases of EPE. However, in most cases of EPE, no history or evidence of direct or indirect exposure to pigs or pig feces has been reported. Further, multilocus VNTR profile of pig and equine isolates differ greatly.[22] A recent experimental study has

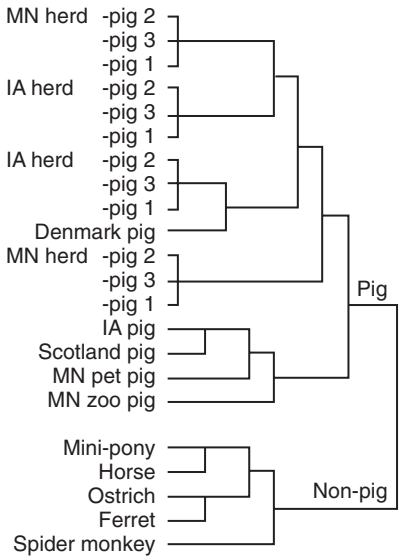

Figure 34-1 Dendrogram showing representative variable number tandem repeat (VNTR) sequence relationships between *Lawsonia intracellularis* isolates from various animal species and geographic sources. *MN*, Minnesota; *IA*, Iowa.

shown host specificity for *L. intracellularis* isolates cultured from pig or horse intestines.[27] The study showed that clinical signs, longer periods of shedding, and stronger serologic immune responses were observed in animals infected with species-specific isolates. Previous studies have shown that a variety of wild and domestic animals, including dogs, cats, rabbits, opossums, skunks, mice, and coyotes, can shed *L. intracellularis* on farms with diagnosed EPE cases.[28-29] On a recently identified farm in California endemic for EPE, 7.5% of fecal samples and 27% of serum samples from cottontail rabbits tested PCR positive and seropositive for *L. intracellularis*, respectively.[29] Of interest was that on this farm, a large population of cottontail rabbits lived in the hay barn and had direct access to the hay fed to the horses. An epidemiologic investigation on this farm showed rabbit feces on top of hay bales but also in the feeders of the weanling foals, suggesting that the foals developing EPE were likely exposed to *L. intracellularis* via the oral ingestion of infected rabbit feces. Similar to rodents, lagomorphs may represent an effective reservoir/amplifier host because of their large population, their close contact to horses, and their short reproductive cycle. It still remains to be determined how *L. intracellularis* became endemic in the rabbit population on this farm.

Feco-oral transmission of *L. intracellularis* has been documented in naïve foals housed with clinically infected foals experimentally challenged with an equine isolate of *L. intracellularis*.[30] A recent study demonstrated that feces from rabbits experimentally infected with an equine isolate of *L. intracellularis* served as infectious material to weanling foals.[31] Although infected rabbits and foals remained asymptomatic, infection was supported by fecal shedding of *L. intracellularis* and detection of specific antibodies to *L. intracellularis*. Although the natural infectious dose for foals has not been determined, pigs receiving as low as 10^5 *L. intracellularis* have been shown to develop infection.[32] Recent work suggested that 1 g of infectious feces would suffice to deliver this challenge dose.[25] Likely, the initial transmission of *L. intracellularis* occurs via the accidental ingestion of infectious feces from one of the described or as yet undetermined amplifiers/hosts. Amplification of *L. intracellularis* and environmental contamination leading to exposure rates of up to 100% of resident foals are likely to occur secondary to the shedding of large quantities of *L. intracellularis* from either clinically or subclinically infected foals.

In piglets, large group size, weaning, transportation, diet change, and mixing have been associated with clinical disease.[1] Predisposing factors, such as the stress of weaning, overcrowding, decline in *L. intracellularis*-specific colostral antibodies, endoparasitism, and introduction of new animals, have been suggested in the development of EPE in foals.[7] In pigs, infection and fecal shedding of *L. intracellularis* may persist for as long as 12 weeks.[33] In contrast, the horse may have a shorter duration of infectivity; experimentally infected foals showed an onset and duration of fecal shedding between 10 and 14 days and 17 and 27 days, respectively.[30,34] *Lawsonia intracellularis* can survive in environmental conditions for 1 to 2 weeks at 5° C to 15° C.[35]

Pathogenesis

The pathogenesis of EPE has remained poorly investigated, and most of the information available has been extrapolated from experimentally infected hamsters, pigs, and rabbits. Comprehensive studies of lesion development and evolution have been conducted in pigs[36] and hamsters.[37] Morphologic studies of early lesions in experimentally infected animals indicate that enterocyte hyperplasia is directly preceded by the presence of the intracellular organism.[36,37] In vivo, the onset of hyperplasia associated with proliferative enteropathy follows an increase in numbers of intracellular *L. intracellularis* in enterocytes. Likewise, resolution of the lesions is closely related to disappearance of the intracellular organisms, indicating a correlation between the two events.[1] The means by which *L. intracellularis* produces hyperplasia is unknown. No other cytopathologic effects on infected enterocytes are seen in vivo or in vitro. Inflammation becomes evident in later-stage lesions and is not characteristic of the primary lesion.

Convalescent pigs have a degree of immunity to reinfection.[32] Animals challenged a second time, after cessation of fecal shedding, were evaluated clinically and their feces were tested by PCR to detect shedding. Animals previously infected did not shed detectable numbers of *L. intracellularis* and had no clinical signs. The cell-mediated immune response may be an important feature in protecting animals from reinfection with *L. intracellularis*. Descriptive immunocytologic studies of intestinal tissue sections of pigs affected by proliferative enteropathy reveal a mild infiltration of cytotoxic T cells, macrophages, and B lymphocytes carrying major histocompatibility complex (MHC) class II structure at the beginning of the cell-mediated immune response.[38]

Immunohistochemical studies of intestinal sections of naturally infected pigs also demonstrated a large accumulation of immunoglobulin A (IgA) in the apical cytoplasm of proliferating enterocytes.[38] Further, interferon gamma (IFN-γ) is produced by peripheral blood mononuclear cells (PBMCs) of both pigs and horses following specific stimulation,[33,39,40] and IgA is detected in intestinal lavages of challenged pigs.[32] Similarly, IFN-γ played a role in limiting intracellular infection and increased cellular proliferation in experimentally infected mice.[41]

Clinical Findings

There are characteristic signalment, seasonality, clinical signs, and blood work abnormalities associated with EPE. The disease is generally manifested in foals less than 1 year of age, and in North America, EPE is often seen between August and January.[16] Although the disease is commonly seen in weanling foals 4 to 7 months of age, cases of EPE have been seen in young adults (Pusterla, personal communication). Lethargy, anorexia, fever (>38.5° C [101.3° F]), peripheral edema (ventrum, sheath, throatlatch, and distal limbs; Figs. 34-2 and 34-3), weight loss

Figure 34-2 Ventral and distal limb edema in a 7-month-old Thoroughbred filly with equine proliferative enteropathy (EPE).

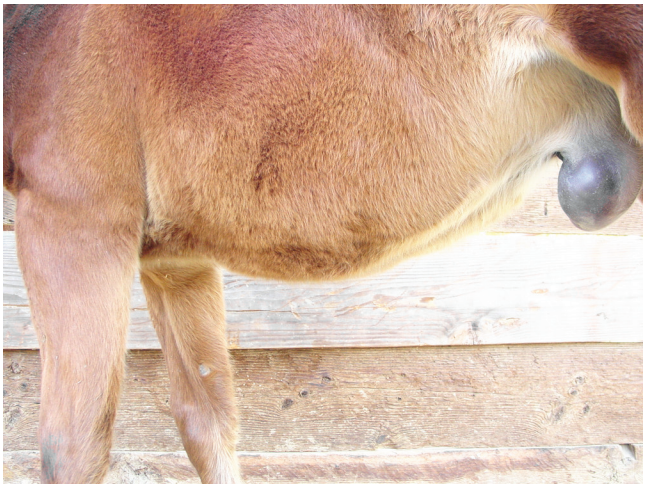

Figure 34-3 Ventral and sheath edema in an 8-month-old Quarter Horse colt with equine proliferative enteropathy (EPE).

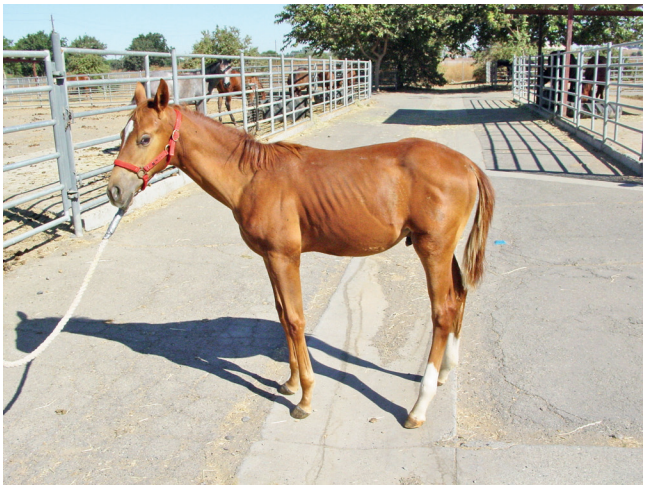

Figure 34-4 Severe weight loss in a 5-month-old Quarter Horse colt with equine proliferative enteropathy (EPE).

(Fig. 34-4), colic, and diarrhea (Fig. 34-5) are among the most common clinical findings in affected foals. Early clinical signs are generally nonspecific and include mild depression, partial anorexia, and fever. Although diarrhea is commonly seen in affected foals and can vary from cow pie to watery, some affected foals may have normal fecal character. Foals with EPE may also have concurrent disorders such as respiratory tract infections, gastric ulcerations, and intestinal parasitism. Signs of EPE may resemble those of more common gastrointestinal disorders such as parasitism; bacterial infections (*Clostridium* spp., *Salmonella* spp., *Rhodococcus equi*, *Neorickettsia risticii*); rotavirus, coronavirus; ulcerations; sand accumulation; intestinal obstruction; and intoxication with plants, chemicals, and pharmacologic agents such as nonsteroidal antiinflammatory drugs (NSAIDs) or antimicrobials. Similar to pigs, the disease can be subclinical in foals. Subclinical disease is characterized by a self-limiting and transient decrease of total serum protein concentration coupled with decreased daily weight gain when compared to unaffected foals.[30,34] It will remain to be determined if growth retardation or unthriftiness are associated with subclinical infection.

The most consistent laboratory finding of clinical EPE is hypoproteinemia caused by hypoalbuminemia. Total protein is

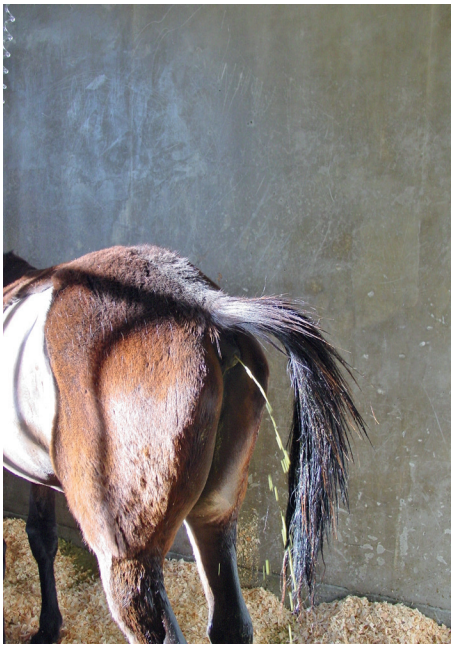

Figure 34-5 Diarrhea in an 8-month-old Thoroughbred colt with equine proliferative enteropathy (EPE).

generally less than 5.0 g/dL, and albumin is usually less than 2.0 g/dL. In a recent case report,[16] hypoalbuminemia was the only consistent clinicopathologic abnormality of 57 affected foals, with albumin concentrations ranging from 0.9 to 3.3 g/dL (normal reference range 2.7 to 4.2 g/dL). The exact mechanisms by which hypoalbuminemia develops in affected foals has not been investigated. It appears that a combination of decreased feed intake, coupled with malabsorption and protein-losing enteropathy as a result of the proliferative nature of the disease may represent likely mechanisms by which low albumin occurs.[42] Affected foals may also demonstrate nonspecific blood abnormalities such as anemia or hemoconcentration, leukocytosis or neutropenia, hyperfibrinogenemia, increased activity of muscle enzymes, and electrolyte abnormalities (hypocalcemia, hypochloremia, and hyponatremia). Urine analysis to rule out protein-losing nephropathy and cytologic evaluation of abdominal fluid to rule out protein lost to a third space are generally unremarkable.

Diagnosis

A presumptive diagnosis of EPE is generally made based on the age of the affected animal, clinical signs, hypoproteinemia/hypoalbuminemia, presence of thickened small intestinal loops on ultrasonographic evaluation, and ruling out other causes of enteropathy and protein losses. Abdominal ultrasonography, although not very sensitive, may show segments of thickened small intestine (Fig. 34-6) and excessive abdominal fluid. In these cases, abdominocentesis will yield a noninflammatory transudate. An antemortem diagnosis is generally confirmed via PCR detection of *L. intracellularis* in feces or rectal swab and/or serology.

It is essential to combine both molecular and serologic diagnostic testing because these modalities have high analytical specificity but variable sensitivity, depending on the situation. Negative PCR results can be expected if the fecal samples are collected from foals with prior antimicrobial treatment or

during advanced disease stage when *L. intracellularis* organisms are no longer expected in the feces. Negative serologic results can be expected in the early stage of the disease when humoral immune responses are not yet strong enough to be detectable by serology. Further, differences in sensitivity among different PCR and serologic assays can lead to divergent results. Among PCR assays, the use of real-time platform has been shown to yield the best sensitivity and to reduce the likelihood of cross- or carry-over contamination (i.e., false-positive results).[43-45] Several serologic assays, including indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), and immunoperoxidase monolayer assay (IPMA), have all been validated and established for pigs.[46-49] However, a preliminary comparative study using equine serum samples has shown that IPMA is the most accurate of all serologic tests to determine the presence of specific anti-*Lawsonia intracellularis* antibodies in foals with EPE (Gebhart, personal communication).

Based on clinical observations, it appears that the exposure rate to *L. intracellularis* is higher than the clinical attack rate; however, assuming that index cases are only the apex tip of the pyramid, it is always advisable to test herdmates to determine their exposure and clinical status. This is best achieved by collecting blood to determine the level of anti-*Lawsonia intracellularis* antibodies by serology and to measure total protein concentration by refractometry. Another more expensive alternative is to measure total protein and/or albumin concentrations by chemical analysis. Polymerase chain reaction testing of feces from healthy herdmates is not advised in this situation because of the expense of testing and low rate of positivity. Also, the results from previous epidemiologic studies show that healthy herdmates rarely shed detectable *L. intracellularis*.[50,51] Daily syndromic surveillance of all herdmates is also recommended in order to recognize early stages of disease. This is best achieved via daily physical examination, including rectal temperature and the regular assessment of weight, allowing the calculation of daily weight gain. A positive titer by IPMA (≥60) in a healthy herdmate with no hypoproteinemia should be viewed as past exposure with no apparent disease or possibly early, not yet clinically apparent EPE. Seropositive or seronegative clinically healthy herdmates with hypoproteinemia (<5.0 g/dL) or hypoalbuminemia (<3.0 g/dL) should undergo further diagnostic testing (white blood cell count, abdominal ultrasound examination, fecal PCR) to determine if *L. intracellularis* infection is the cause of the hypoproteinemia. Treating foals with suspected EPE based only on clinical findings and hypoproteinemia/hypoalbuminemia is not recommended because of the risk associated with the use of antimicrobials. Healthy seronegative herdmates with no hypoproteinemia should continue to be monitored daily for clinical signs and monthly or bimonthly for hypoproteinemia and/or hypoalbuminemia and detectable antibodies to *L. intracellularis*. Any foal developing clinical signs of EPE should undergo a thorough diagnostic workup. Further, clinically affected foals or foals with suspected clinical EPE should be separated from the rest of the healthy herdmates to decrease environmental contamination until their shedding status has been determined by PCR. It has been previously shown that experimentally infected foals start shedding *L. intracellularis* 5 to 17 days prior to developing hypoproteinemia and clinical signs.[30] It is this prodromal stage of subclinically infected foals that is likely responsible for the environmental contamination and exposure of susceptible foals.

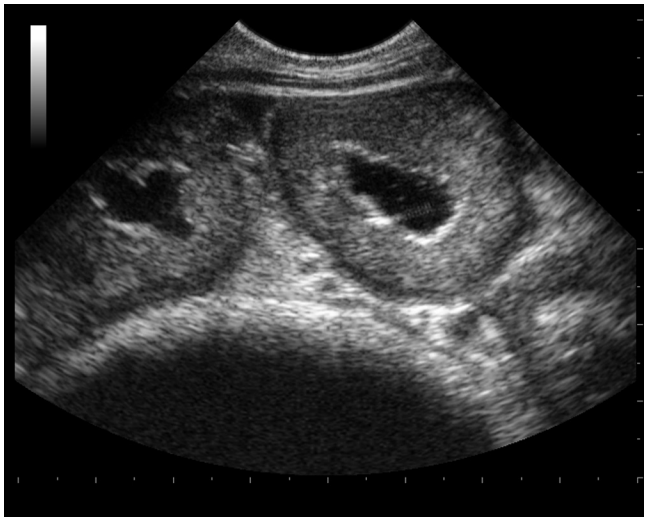

Figure 34-6 Ultrasound image showing thickened section of small intestinal wall in a 5-month-old Quarter Horse filly with equine proliferative enteropathy (EPE). The wall thickness measured 4.3 mm (normal wall thickness ≤3 mm).

Pathologic Findings

Lesions are most commonly seen in the ileum, near the ileal-cecal junction, and appear as a thickening of the mucosa. Gross lesions are not evident in all cases of EPE and may often be overlooked. Intestines show an irregular, patchy subserosal edema. The ileal mucosa is thickened with deep folds and chronically affected animals may have patches of pseudomembrane covering the mucosa (Fig. 34-7). Hypertrophy and thickening of the muscularis mucosa may occur in chronically affected or recovering animals (Fig. 34-8). Histologically,

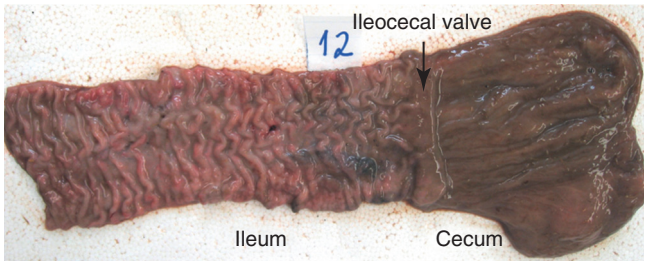

Figure 34-7 Gross lesions of equine proliferative enteropathy (EPE). Ileal-cecal junction of an affected 5-month-old foal showing thickened ileal mucosa with a corrugated appearance.

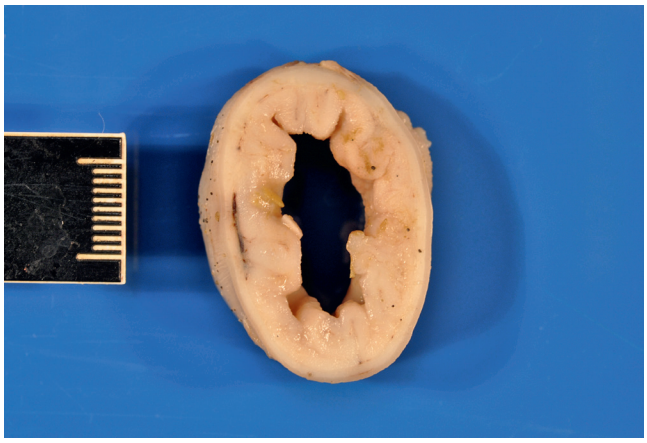

Figure 34-8 Cross-section of ileum of an 8-month-old Thoroughbred foal with equine proliferative enteropathy (EPE) showing diffusely thickened intestinal wall.

adenomatous proliferation occurs among the epithelial cells in the crypts of the small intestine, in association with the presence of curved, intracellular bacteria in the apical cytoplasm of these enterocytes.[2,3,7] Severe EPE is diagnosed by the demonstration of hyperplasia of the crypt glands with an increased number of mitotic figures and marked reduction or absence of goblet cells in routine hematoxylin and eosin preparations (Fig. 34-9); however, for visualization of the bacteria in the cytoplasm of enterocytes, special stains are necessary. The histologic lesions of PE are unique and inflammation is not normally a hallmark of disease. Warthin-Starry silver stain allows the detection of the bacteria in histologic sections, improving the diagnostic sensitivity, but the technique has limitations when applied to autolyzed and necrotic samples.[1] Immunohistochemistry procedures, using postmortem tissue or biopsy material with an antibody specific for *L. intracellularis*, have been used successfully to diagnose EPE (Fig. 34-10).

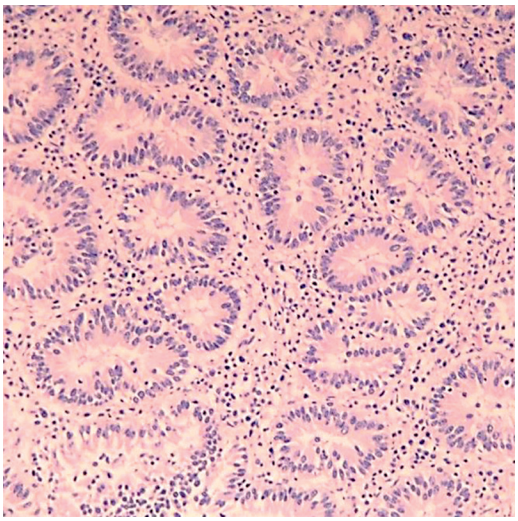

Figure 34-9 Hematoxylin and eosin (H&E)-stained section of small intestine from an 8-month-old foal with equine proliferative enteropathy (EPE) showing marked hyperplasia of crypt glands with lack of goblet cells.

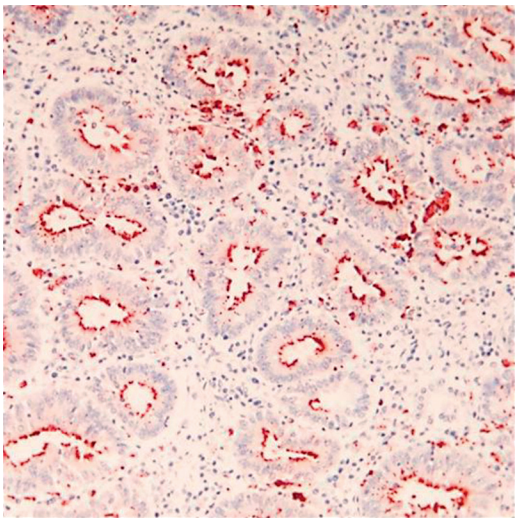

Figure 34-10 Immunohistochemical stained section of small intestine from an 8-month-old foal with equine proliferative enteropathy (EPE). *Lawsonia intracellularis*–specific antibody stains the bacteria lining the apical cytoplasm of the affected crypts *(red areas)*.

Therapy

It is important to treat affected animals early, before lesions become advanced and result in marked weight loss and critically low serum protein values. Treatment of EPE in horses involves the use of antimicrobials such as macrolides, alone or in combination with rifampin, chloramphenicol, oxytetracycline, or doxycycline administered for 2 to 3 weeks. The choice of antimicrobial in the treatment of EPE should take into account the risk of inducing disturbance of the gastrointestinal flora and renal toxicity. This is especially a concern when treating older foals with severe hypoalbuminemia. In addition, supportive care, such as intravenous (IV) fluids, plasma transfusion, parenteral nutrients, and antiulcer drugs are commonly used to treat affected foals. Concurrent medical conditions should also be addressed. Rapid clinical improvement following treatment is to be expected; however, it may take weeks for the hypoproteinemia to resolve. Spontaneous recovery of clinically affected foals has not been documented, and treated foals usually survive the disease. Long-term sequelae have not been reported; however, clinically affected and successfully treated foals sell for an average of 68% of the average price of unaffected foals by the same stallion.[16]

Prevention

The monitoring of a herd with endemic status follows guidelines similar to those for herds with diagnosed index cases. This includes the regular physical evaluation of resident foals and the monthly or bimonthly assessment of total protein concentration and monthly serologic status. Monitoring for exposure to *L. intracellularis* and hypoproteinemia/hypoalbuminemia should begin at least 4 weeks prior to the historic first detection of clinical cases. Monthly data, including concentration of total solids or albumin and weight gains, should be evaluated for each foal and compared to the previous month's data to determine decreasing trends potentially associated with early disease. Recent work performed in central Kentucky has shown a seasonality to EPE cases, with peak cases recorded in November and December.[16] Year-to-year variations, depending on climatic conditions, can be expected; however, most of the EPE cases are seen between August and January in the northern hemisphere, which relates to the age of the foals. Considering the cost of treating a foal with clinical EPE, this monitoring program is cost-effective, especially if concentration of total serum solids can be assessed by farm personnel. The lack of epidemiologic data regarding potential natural reservoir hosts, as well as the lack of information pertaining to the biology of *L. intracellularis*, precludes the institution of any management changes on endemic farms. Early recognition of clinical cases and separating them from the rest of the susceptible foals until full recovery or cessation of fecal shedding appear to be logical biosecurity measures to prevent spread and environmental contamination. Further, maintaining good pest control and preventing nonequine domestic and wild animals access to feed and feeding areas may potentially minimize the risk of disease spread.

Prevention strategies have been best described in pigs using in-feed antimicrobials and a commercially available *L. intracellularis* vaccine.[33,52-54] Recent work has shown that detectable humoral and cellular responses can be measured in foals administered an avirulent live *L. intracellularis* vaccine.[34,55-57] The recently established vaccine protocol has shown that the intrarectal administration of 30 mL of either the lyophilized or the frozen-thawed formulation of the avirulent *L. intracellularis* vaccine given twice, 30 days apart, yielded the strongest

immunologic responses.[55] The *L. intracellularis* vaccine is safe, and the administration well tolerated by foals. Further, the avirulent *L. intracellularis* vaccine has not been associated with the induction of clinical disease in pigs or foals. Fecal shedding for up to 12 days has been documented following intrarectal vaccine administration in foals.[55] Using the previously mentioned protocols, vaccine efficacy has been evaluated in the field and, more recently, under experimental conditions. A field efficacy trial performed on EPE endemic farms in central Kentucky in 2008 showed that vaccinated foals maintained higher daily weight gains and higher total protein concentrations when compared to a nonvaccinated, naturally seroconverted group.[58] Because of the low incidence of disease reported on the study farms, no difference in attack rate between vaccinated and nonvaccinated foals could be determined. The overall decreased disease prevalence in the study population may have been associated with the ongoing vaccine trial on these farms because disease prevalence in central Kentucky did not change in 2009 compared to 2008. Potential explanation of the decreased number of clinical cases was the elimination of so called "super shedders" and possible exposure of nonvaccinated foals to *L. intracellularis* vaccine organism shed in the feces of recently vaccinated foals. Under experimental conditions, weanling foals vaccinated intrarectally with an avirulent live vaccine against *L. intracellularis* were protected against clinical and subclinical EPE following challenge exposure with a virulent *L. intracellularis* isolate of equine origin.[34] This was determined by lack of clinical disease, absence of hypoproteinemia and sonographic abnormalities compatible with EPE, and a significant reduction in *L. intracellularis* fecal shedding in vaccinated foals compared to nonvaccinated foals. Further, average daily weight gains from the vaccinated foals over the entire study period were similar to the control foals and significantly higher when compared to the nonvaccinated foals, highlighting the benefit of the vaccine in the prevention of subclinical disease. The extralabel use of the *L. intracellularis* vaccine should be considered on naïve and endemic farms in an attempt to reduce or prevent EPE. Timing of vaccine administration should again be synchronized with historic disease occurrence. Further, routine monitoring for clinical signs and hypoproteinemia/hypoalbuminemia is still recommended, even when vaccine prophylaxis is used.

The complete reference list is available online at www.equineinfectiousdiseases.com.

References

1. Lawson GH, Gebhart CJ: Proliferative enteropathy. J Comp Pathol 122:77–100, 2000.
2. Duhamel GE, Wheeldon EB: Intestinal adenomatosis in a foal. Vet Pathol 19:447–449, 1982.
3. Williams NM, Harrison LR, Gebhart CJ: Proliferative enteropathy in a foal caused by *Lawsonia intracellularis*-like bacterium. J Vet Diagn Invest 8:254–256, 1996.
4. Frank N, Fishman CE, Gebhart CJ: Lawsonia intracellularis proliferative enteropathy in a weanling foal. Equine Vet J 30:549–552, 1998.
5. Brees DJ, Sondhoff AH, Kluge JP, et al: *Lawsonia intracellularis*-like organism infection in a miniature foal. J Am Vet Med Assoc 215:511–515, 1999.
6. Schumacher J, Schumacher J, Rolsma M, et al: Surgical and medical treatment of an Arabian filly with proliferative enteropathy caused by *Lawsonia intracellularis*. J Vet Intern Med 14:630–632, 2000.
7. Lavoie JP, Drolet R, Parsons D, et al: Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinemia in foals on three breeding farms in Canada. Equine Vet J 32:418–425, 2000.
8. Bihr TP: Protein-losing enteropathy caused by *Lawsonia intracellularis* in a weanling foal. Can Vet J 44:65–66, 2003.
9. McClintock SA, Collins AM: *Lawsonia intracellularis* proliferative enteropathy in a weanling foal in Australia. Aust Vet J 82:750–752, 2004.
10. Deprez P, Chiers K, Gebhart CJ, et al: *Lawsonia intracellularis* infection in a 12-month-old colt in Belgium. Vet Rec 157:774–776, 2005.
11. Dauvillier J, Picandet V, Harel J, et al: Diagnostic and epidemiological features of *Lawsonia intracellularis* enteropathy in 2 foals. Can Vet J 47:689–691, 2006.
12. Sampieri F, Hinchcliff KW, Toribio RE: Tetracycline therapy of *Lawsonia intracellularis* enteropathy in foals. Equine Vet J 38:89–92, 2006.
13. Wuersch K, Huessy D, Koch C, et al: *Lawsonia intracellularis* proliferative enteropathy in a filly. J Vet Med A Physiol Pathol Clin Med 53:17–21, 2006.
14. Feary DJ, Gebhart CJ, Pusterla N: *Lawsonia intracellularis* proliferative enteropathy in a foal. Schweiz Arch Tierheilkd 149:129–133, 2007.
15. McGurrin MK, Vengust M, Arroyo LG, et al: An outbreak of *Lawsonia intracellularis* infection in a standardbred herd in Ontario. Can Vet J 48:927–930, 2008.
16. Frazer ML: *Lawsonia intracellularis* infection in horses: 2005-2007. J Vet Intern Med 22:1243–1248, 2008.
17. Merlo JL, Sheats MK, Elce Y, et al: Outbreak of *Lawsonia intracellularis* on a standardbred farm in North Carolina. Equine Vet Educ 21:179–182, 2009.
18. Guimarães-Ladeira CV, Palhares MS, Oliveira JS, et al: Faecal shedding and serological cross-sectional study of *Lawsonia intracellularis* in horses in the state of Minas Gerais, Brazil. Equine Vet J 41:593–596, 2009.
19. Shimizu C, Shibahara T, Takai S, et al: *Lawsonia intracellularis* and virulent *Rhodococcus equi* infection in a Thoroughbred colt. J Comp Pathol 143:303–308, 2010.
20. Van Den Wollenberg L, Butler CM, Houwers DJ, et al: *Lawsonia intracellularis*-associated proliferative enteritis in weanling foals in the Netherlands. Tijdschr Diergeneeskd 136:565–570, 2011.
21. Cooper DM, Gebhart CJ: Comparative aspects of proliferative enteritis. J Am Vet Med Assoc 212:1446–1451, 1998.
22. Al-Ghamdi M: Characterization of proliferative enteropathy in horses, PhD Thesis, 2003, University of Minnesota.
23. Jordan DM, Knittel JP, Schwartz KJ, et al: A *Lawsonia intracellularis* transmission study using a pure culture inoculated seeder-pig sentinel model. Vet Microbiol 104:83–90, 2004.
24. Bednar V: Detection of *Lawsonia intracellularis* in mice captured in pig farms with the occurrence of porcine proliferative enteropathy. In Proceedings of the 19th International Pig Veterinary Society Congress, Copenhagen, 2006, p 180.
25. Collins AM, Fell S, Pearson H, et al: Colonisation and shedding of *Lawsonia intracellularis* in experimentally inoculated rodents and in wild rodents on pig farms. Vet Microbiol 150:384–388, 2011.
26. Friedman M, Bednár V, Klimes J, et al: *Lawsonia intracellularis* in rodents from pig farms with the occurrence of porcine proliferative enteropathy. Lett Appl Microbiol 47:117–121, 2008.
27. Vannucci FA, Pusterla N, Mapes SM, et al: Evidence of host adaptation in *Lawsonia intracellularis* infections. Vet Microbiol. 162:265–269, 2013.
28. Pusterla N, Mapes S, Rejmanek D, et al: Detection of *Lawsonia intracellularis* by real-time PCR in the feces of free-living animals from equine farms with documented occurrence of equine proliferative enteropathy. J Wildl Dis 44:992–998, 2008.
29. Pusterla N, Mapes S, Gebhart C: Further investigation of exposure to *Lawsonia intracellularis* in wild and feral animals captured on horse properties with equine proliferative enteropathy. Vet J 194;253–255, 2012.
30. Pusterla N, Wattanaphansak S, Mapes S, et al: Oral infection of weanling foals with an equine isolate of *Lawsonia intracellularis*, agent of equine proliferative enteropathy. J Vet Intern Med 24:622–627, 2010.
31. Pusterla N, Sanchez-Migallon Guzman D, Vannucci FA, et al: Transmission of *Lawsonia intracellularis* to weanling foals using feces from experimentally infected rabbits, unpublished manuscript, 2012.
32. Collins AM, Love RJ: Re-challenge of pigs following recovery from proliferative enteropathy. Vet Microbiol 120:381–386, 2007.
33. Guedes RM, Gebhart CJ: Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic isolate or attenuated vaccine strain of *Lawsonia intracellularis*. Vet Microbiol 91:135–145, 2003.
34. Pusterla N, Vannucci FA, Mapes MS, et al: Evaluation of an avirulent live vaccine against *Lawsonia intracellularis* in the prevention of proliferative enteropathy in experimentally infected weanling foals. Am J Vet Res 73:741–746, 2012.
35. Collins A, Love RJ, Pozo J, et al: Studies on the ex vivo survival of *Lawsonia intracellularis*. J Swine Health Prod 8:211–215, 2000.
36. Guedes RM: Porcine proliferative enteropathy: diagnosis, immune response and pathogenesis, PhD Thesis, 2002, University of Minnesota.
37. Jacoby RO: Transmissible ileal hyperplasia of hamsters. Am J Pathol 91:433–444, 1978.
38. McOrist S, MacIntyre N, Stokes CR, et al: Immunocytological responses in porcine proliferative enteropathies. Infect Immun 60(10):4184–4191, 1992.
39. Pusterla N, Mapes S, Gebhart C: *Lawsonia intracellularis*-specific interferon γ gene expression by peripheral blood mononuclear cells in vaccinated and naturally infected foals. Vet J 192(2):249–251, 2012.
40. Page AE, Loynachan AT, Bryant U, et al: Characterization of the interferon gamma response to *Lawsonia intracellularis* using an equine proliferative enteropathy challenge (EPE) model. Vet Immunol Immunopathol 143:55–65, 2011.
41. Smith DG, Mitchell SC, Nash T, et al: Gamma interferon influences intestinal epithelial hyperplasia caused by

Lawsonia intracellularis infection in mice. Infect Immun 68:6737–6743, 2000.
42. Wong DM, Alcott CJ, Sponseller BA, et al: Impaired intestinal absorption of glucose in 4 foals with *Lawsonia intracellularis infection*. J Vet Intern Med 23:940–944, 2009.
43. Nathues H, Holthaus K, Beilage E: Quantification of *Lawsonia intracellularis* in porcine faeces by real-time PCR. J Appl Microbiol 107:2009–2016, 2009.
44. Richter B, Ladinig A, Nedorost N, et al: A TaqMan quantitative polymerase chain reaction assay for the detection of *Lawsonia intracellularis* in fecal and tissue samples from pigs. J Vet Diagn Invest 22:70–73, 2010.
45. Pusterla N, Mapes S, Johnson C, et al: Comparison of feces versus rectal swabs for the molecular detection of *Lawsonia intracellularis* in foals with equine proliferative enteropathy. J Vet Diagn Invest 22:741–744, 2010.
46. Guedes RM, Gebhart CJ, Winkelman NL, et al: Comparison of different methods for diagnosis of porcine proliferative enteropathy. Can J Vet Res 66:99–107, 2002.
47. Guedes RM, Gebhart CJ, Winkelman NL, et al: A comparative study of an indirect fluorescent antibody test and an immunoperoxidase monolayer assay for the diagnosis of porcine proliferative enteropathy. J Vet Diagn Invest 14:420–423, 2002.
48. Boesen HT, Jensen TK, Møller K, et al: Evaluation of a novel enzyme-linked immunosorbent assay for serological diagnosis of porcine proliferative enteropathy. Vet Microbiol 109:105–112, 2005.
49. Wattanaphansak S, Asawakarn T, Gebhart CJ, et al: Development and validation of an enzyme-linked immunosorbent assay for the diagnosis of porcine proliferative enteropathy. J Vet Diagn Invest 20:170–177, 2008.
50. Pusterla N, Higgins JC, Smith P, et al: Epidemiological survey on farms with documented occurrence of equine proliferative enteropathy due to *Lawsonia intracellularis*. Vet Rec 163:156–158, 2008.
51. Pusterla N, Jackson R, Wilson R, et al: Temporal detection of *Lawsonia intracellularis* using serology and real-time PCR in Thoroughbred horses residing on a farm endemic for equine proliferative enteropathy. Vet Microbiol 136: 173–176, 2009.
52. Kroll JJ, Roof MB, McOrist S: Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of *Lawsonia intracellularis*. Am J Vet Res 65:559–565, 2004.
53. Almond PK, Bilkei G: Effects of oral vaccination against *Lawsonia intracellularis* on growing-finishing pig's performance in a pig production unit with endemic porcine proliferative enteropathy (PPE). Dtsch Tierarztl Wochenschr 113:232–235, 2006.
54. McOrist S, Smits RJ: Field evaluation of an oral attenuated *Lawsonia intracellularis* vaccine for porcine proliferative enteropathy (ileitis). Vet Rec 161:26–28, 2007.
55. Pusterla N, Hilton H, Wattanaphansak S, et al: Evaluation of the humoral immune response and fecal shedding in weanling foals after oral and intra-rectal administration of an avirulent live vaccine of *Lawsonia intracellularis*. Vet J 182:458–462, 2009.
56. Puterla N, Collier J, Mapes SM, et al: Effects of administration of an avirulent live vaccine of *Lawsonia intracellularis* on mares and foals. Vet. Rec 164:783–785, 2009.
57. Pusterla N, Jackson R, Mapes SM, et al: *Lawsonia intracellularis:* humoral immune response and fecal shedding in weanling foals following intra-rectal administration of frozen-thawed or lyophilized avirulent vaccine. Vet J 186:110–112, 2010.
58. Nogradi N, Slovis NM, Gebhart CJ, et al: Evaluation of the field efficacy of an avirulent live *Lawsonia intracellularis* vaccine in foals. Vet J 192(3):511–513, 2012.