How to Monitor and Prevent the Occurrence of *Lawsonia intracellularis* Infection in Weanling Foals From Farms With Endemic or Sporadic Occurrence of Equine Proliferative Enteropathy

Nicola Pusterla, DVM, PhD, Diplomate ACVIM*; Connie Gebhart, PhD; and Nathan M. Slovis, DVM, Diplomate ACVIM

1. Introduction

*Lawsonia intracellularis* is the etiologic agent of the recently recognized and emerging intestinal disease in foals, called equine proliferative enteropathy (EPE). *L. intracellularis* is an obligate intracellular, curved, Gram-negative bacterium that resides freely within the apical cytoplasm of infected intestinal enterocytes. It causes proliferation of the affected enterocytes, resulting in a thickened small and sometimes large intestine, which eventually leads to malabsorption.1

Since the first report of EPE in 1982, many more reports of sporadic cases and outbreaks on breeding farms have been described.2–18 In the last few years, reported cases of EPE have been increasing, primarily in post-weaning foals and occasionally in adult horses. The disease has reached a worldwide occurrence and has been reported in the United States, Canada, Europe, Australia, Brazil, South Africa, and Japan.

Clinical signs associated with EPE include fever, lethargy, anorexia, peripheral edema, diarrhea, colic, and weight loss.19 That said, early signs can be subtle and only include partial anorexia, mild fever, and peripheral edema. Similar to what has been reported in pigs,20 foals also develop a subclinical disease characterized by lower daily weight gain.21 The diagnosis of EPE may be challenging and relies on the presence of hypoproteinemia caused by hypoalbuminemia, thickening of segments of the small intestinal wall observed on abdominal ultrasonography, positive serology, and molecular detection of *L. intracellularis* in feces.22 It is important to treat affected animals early, before lesions become advanced, resulting 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, oxytetracy-
cyccline, 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 a concern especially when treating older foals or adults with severe hypoaalbuminemia. In addition, supportive care such as intravenous fluids, colloids, plasma transfusion, parenteral nutrients, and anti-ulcer drugs are commonly used to treat affected foals. Concurrent medical conditions should also be addressed. Rapid clinical improvement after treatment is to be expected; however, it may take weeks for the hypoproteinemia to resolve. Spontaneous recovery of clinically infected foals has not been well documented, and treated foals usually survive the disease. Long-term sequelae have not been reported; however, affected horses sold as yearlings an average of 68% less than other yearlings by the same sire.

Although the clinical entity, diagnostic workup, and treatment of EPE are well established and described, preventive measures for the disease have remained largely unaddressed. Such strategies have been developed for pigs because the chronic form of swine proliferative enteropathy is associated with high economic losses caused by uneven weight gain and mortality. At herd level in pigs, proliferative enteropathy is mainly controlled by antimicrobial treatment and vaccination with an attenuated live oral vaccine. Recent work in foals with the avirulent live L. intracellularis vaccine administered intra-rectally has shown that the vaccine is safely administered and triggers humoral and cell-mediated immune responses similar to natural infection. The objectives of this report are to describe protocols used to monitor the occurrence of L. intracellularis infection on farms with endemic or sporadic EPE and to present feasible and affordable preventive measures to minimize or reduce the incidence of EPE.

2. Materials and Methods

Diagnosis

A presumptive diagnosis of EPE is generally made on the basis of age of the affected animal (3 to 13 months), clinical signs, hypoproteinemia (<5.0 g/dl)/ hypoaalbuminemia (<3.0 g/dl), presence of thickened small intestinal loops on ultrasonographic evaluation, and ruling out other causes of enteropathy and protein losses. An antemortem diagnosis is generally confirmed via polymerase chain reaction (PCR) detection of L. intracellularis in feces or rectal swab and/or serology. It is essential to combine both the molecular and serologic diagnostic testing because these modalities have high analytical specificity but variable sensitivity. 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 serological 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 serological assays can lead to divergent results. Among PCR assays, the use of a real-time platform has been shown to yield the best sensitivity and to reduce the likelihood of cross-over or carry-over contamination (i.e., false-positive results). Several serological 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. However, a preliminary comparative study using equine serum samples has shown that the IPMA is the most accurate of all serological tests to determine the presence of anti-L. intracellularis antibodies in foals with EPE. To the author’s knowledge, there are only two diagnostic laboratories in the United States that offer both PCR and IPMA testing for L. intracellularis.

Monitoring of Herd Status After Diagnosis of an Initial Index Case

Based on clinical observations, it appears that the exposure rate to L. intracellularis is higher than the clinical attack rate. That said, and assuming that index cases are only the 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-L. intracellularis antibodies by IPMA and to measure total protein concentration by refractometry. Another more expensive alternative is to measure total protein and/or albumin concentrations by chemical analysis. PCR testing of feces from healthy herdmates is not advised in this situation because of the costs of testing. Also, the results from previous epidemiological studies show that healthy herdmates rarely shed detectable L. intracellularis. Daily clinical monitoring of all herdmates is also recommended to recognize early stage of disease. This is best achieved through daily physical examination, including temperature and the regular assessment of weight, allowing the calculation of daily weight gain. A positive IPMA titer (>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. In the latest situation, the foal’s clinical parameters and total protein concentration should be regularly monitored. Seropositive or seronegative clinically healthy herdmates with hypoproteinemia (<5.0 g/dl) or hypoalbuminemia (<3.0 g/dl) should undergo further diagnostic testing (cell blood count, abdominal ultrasound examination, fecal PCR) to determine if L. intracellularis infection is the cause of the hypoproteinemia and to rule out other enteric or nonenteric differential diagnosis.
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 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 before developing hypoproteinemia and clinical signs.\(^{21}\) This prodromal stage of subclinically infected foals probably is responsible for the environmental contamination and the exposure of susceptible foals. Previous work has shown that *L. intracellularis* is likely to survive in environmental conditions for 1 to 2 weeks at 5° to 15° C.\(^{40}\)

**Monitoring a Herd With Endemic Status**

The monitoring of a herd with endemic status follows similar guidelines as 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 serological status. Monitoring for exposure to *L. intracellularis* and hypoproteinemia/hypoalbuminemia should begin at least 4 weeks prior to the historical first detection of clinical cases. Monthly data of concentration of total solids or albumin concentration and weight gains should be evaluated for each foal and compared with the previous month 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.\(^{15}\) Year-to-year variations, depending on the climatic conditions, can be expected; however, most of the EPE cases are seen between August and January in the northern hemisphere. Considering the cost of treating a clinically affected foal with EPE, this monitoring program is cost-effective, especially if concentration of total serum solids can be performed by farm personal. Because of an increasing demand in herd monitoring, several laboratories are offering dual blood analysis (concentration of total solids or albumin concentration and IPMA) at competitive prices, especially when bulk samples are submitted.\(^{b,c,d}\) The lack of epidemiological data regarding potential natural reservoir hosts and the lack of information pertaining to the biology of *L. intracellularis* preclude 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 appears to be a logical biosecurity measure to prevent spread and environmental contamination. Further, maintaining good pest control and preventing non-equine domestic and wild animals to gain access to feed and feeding areas may potentially minimize the risk of disease spread.

**Prevention of EPE**

In piglets, large group size, weaning, transportation, diet change, and mixing have been associated with increased susceptibility to clinical disease.\(^{20}\) Predisposing factors such as stress of weaning, parasitism, and underlying diseases have been suggested in the development of EPE in foals.\(^{10}\) It is therefore advisable to minimize the stress of weaning and to manage and control heavy parasite burden in susceptible animals.

Prevention strategies have been best described in pigs using in-feed antimicrobials and a commercially available *L. intracellularis* vaccine.\(^{24–27}\) Recent work has shown that detectable humoral and cellular responses can be measured in foals administered an avirulent live *L. intracellularis* vaccine.\(^{28–30}\) The vaccine protocol recently established has shown that the intra-rectal 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 immunological responses.\(^{9}\) The *L. intracellularis* vaccine has been shown to be safe and the administration well tolerated by the foals. Further, the avirulent *L. intracellularis* vaccine has not been associated with the induction of clinical disease in pigs or foals. Fecal shedding up to 12 days has been documented after intra-rectal vaccine administration in foals.\(^{28}\) Using the above-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 with a nonvaccinated, naturally seroconverted group.\(^{41}\) 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, as disease prevalence in Central Kentucky did not change in 2009 compared with 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, weaning foals vaccinated intra-rectally with an avirulent live vaccine against *L. intracellularis* were protected against clinical and subclinical EPE after challenge exposure with a virulent *L.
intracellularis isolate of equine origin. This was determined by lack of clinical disease, absence of hypoproteinemia and ultrasonographic abnormalities compatible with EPE, and a significant reduction in L. intracellularis fecal shedding in vaccinated foals compared with 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 with the nonvaccinated foals, highlighting the benefit of the vaccine in the prevention of subclinical disease. The extra-label 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 historical disease occurrence. Further, routine monitoring for clinical signs and hypoproteinemia/hypoalbuminemia is still recommended even when vaccine prophylaxis is used. The costs of the vaccine are definitively a limiting factor for its routine use on endemic farms. Depending on the formulation (lyophilized versus frozen-thawed), the price per vaccine milliliter ranges from US $1.00 to $1.50. Future studies will be needed to further refine the vaccine protocol and determine if a single dose of a lower vaccine volume still confers protection.

3. Results

Over the past 5 years, herd monitoring after the initial index case has been performed by our research group on 16 horse farms in California, Texas, Maryland, Minnesota, Iowa, and Wisconsin. On each of these farms, blood samples were collected within 10 to 14 days after the confirmation of a clinical case of EPE. Ten farms had 1 single index case and 6 farms had 2 cases. The number of clinically unaffected herdmates ranged from 2 to 98 (median, 10.5 foals). A total of 359 serum samples were tested for concentration of total solids and antibodies against L. intracellularis. Based on the presence of hypoproteinemia (< 5.0 g/dl) and a positive titer against L. intracellularis by IPMA, an additional 19 asymptomatic EPE cases were found. The number of asymptomatic EPE cases ranged from 1 to 5 and originated from 8 different farms. The seroprevalence of the 16 weanling groups ranged from 0% to 100% (Table 1).

Continuous monitoring on endemic herds has been performed on 2 farms (farm 1 and 2) from California and 1 farm (farm 3) from Central Kentucky. These were all larger Thoroughbred breeding farms with 40 to 91 resident weanling foals. Sample collection was performed year-round on farm 1, from October to January on farm 2, and from September to December on farm 3. On all 3 farms, the seroprevalence increased during the winter months. Despite the continuous monitoring, no subclinical foals were found on farms 1 and 2, although no foals were diagnosed with EPE during the study periods. This was in sharp contrast to farm 3, which had 6 clinical cases and 14 subclinical cases.

Field vaccine data are available from farm 3, which elected to vaccinate all their foals in 2010. The 40 resident foals were vaccinated intra-rectally with the avirulent L. intracellularis vaccine during the first week of September and again during the first week of October. Concurrent continuous monitoring of all foals was performed from September through January. One clinical and no subclinical EPE cases were detected during the 2010 foaling season. Ironically, the only clinical case occurred in August before the first L. intracellularis vaccine administration.

4. Discussion

EPE is a true emerging disease, with increasing numbers of cases reported each year. It also appears that an increasing number of horse farms are becoming endemic for this disease. The progressive nature of EPE makes this disease a prime candidate to be monitored on endemic herds. Recognizing EPE in the subclinical stage may prevent sufferance and mortality, decrease costs of treatment, and potentially increase short-term revenue (sales revenue as yearlings) by preventing severe proliferative changes within the small intestine of affected animals. Monitoring strategies combine daily physical evaluation of foals to detect subtle clinical signs with regular measurement of serum protein/albunmin concentration, because hypoproteinemia/hypoalbuminemia is a reliable clinicopath-
ological hallmark of EPE. However, because of the nonspecific nature of the clinical signs and hypoproteinemia/hypoalbuminemia, one must further pursue diagnostics to rule out other possible causes of enteritis and hypoproteinemia. Random and sporadic occurrences of EPE make control difficult, although, once established on a farm, the disease appears to become endemic. After diagnosis of an EPE case, one should consider screening the remaining weanling population to detect subclinically infected animals. The sequential measurement of serum protein or albumin concentrations appears to be a reliable indicator of disease progression. Although this exercise may appear cumbersome, the authors have been able to detect subclinically infected foals and to initiate successful treatment before the development of clinical signs. Although the treatment of clinically infected foals requires antimicrobial therapy and supportive care for at least 3 weeks, subclinically infected animals clear the infection shortly after initiation of antimicrobial treatment. The authors recommend using doxycycline at 10 mg/kg PO q 12 hours administered for 7 to 10 days for the treatment of subclinical infection. Due to the self-limiting nature of the disease in some cases, one could also consider continuous monitoring of physical findings and serum protein/albumin concentration instead of antimicrobial treatment.

On endemic farms, the regular monitoring of physical findings, total protein, and/or albumin concentration may detect subclinically infected foals and to initiate successful treatment before the development of clinical signs. The monitoring need only be performed during the historical time when EPE has been detected on a specific farm and is generally restricted to the months of August through January in the northern hemisphere. EPE appears to be cost-effectively to monitored, based on the recommended monthly or bimonthly measurement of total protein or albumin concentration and of monthly antibody titers to L. intracellularis. Until new information regarding the epidemiology of this disease is determined, preventive strategies are restricted to the use of an avirulent L. intracellularis vaccine. The use of the L. intracellularis vaccine should be considered on naïve and endemic farms, in an attempt to reduce or prevent EPE.


*Gebhart C. Unpublished data, 2011.*

*V Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, 1333 Gortner Avenue, St. Paul, MN 55108.

*Real-time PCR Research and Diagnostics Core Facility, School of Veterinary Medicine, Department of Medicine and Epidemiology, 3110 Tupper Hall, University of California, One Shields Avenue, Davis, CA 95616. Phone: (530) 752-7991; Fax: (530) 754-6862; e-mail: carjohnson@ucdavis.edu; Website: www.vetmed.ucdavis.edu/vme/taqmanservice/diagnostics.html.*

*Hagyard Laboratory, Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY 40511. Phone: (859) 259-3685; Fax: (859) 258-9652; Website: http://hagyard.com/divisions/laboratory.*


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