Proliferative enteropathy is an emerging disease in horses caused by the obligate intracellular organism *Lawsonia intracellularis*. The disease affects weanling foals and has a sporadic occurrence, although outbreaks on breeding farms have been reported. The disease has been reported in the United States, Canada, Europe, South Africa, Australia, Brazil, and Japan. Clinical signs associated with PE in horses include pyrexia, lethargy, anorexia, peripheral edema, diarrhea, colic, and weight loss. Diagnosis of the disease can be challenging and relies on the presence of hypoproteinemia, thickening of segments of the small intestinal wall (> 3 mm) observed via abdominal ultrasonography, positive results of serologic testing, and molecular detection of *L. intracellularis* in feces.

Although diagnostic testing and treatment methods for PE in horses have been established and are well described, preventive measures for the disease have remained largely unaddressed. Preventive strategies have been developed for pigs because, in that species, PE is...
associated with high economic losses due to uneven body weight gain and death. At the herd level, PE in swine is mainly controlled by antimicrobial treatment and vaccination with an attenuated live oral vaccine. \cite{12} Studies \cite{12-14} of the effect of the avirulent live \textit{L. intracellularis} vaccine strain, which can be shed in the feces of infected foals, showed that the vaccine is safe when administered to foals and triggers variable but measurable humoral immune responses.

The purpose of the study reported here was to evaluate the efficacy of an avirulent live \textit{L. intracellularis} vaccine in immunologically naïve weanling foals experimentally challenged with an equine \textit{L. intracellularis} isolate. The primary objective was to determine whether vaccine administration to weanling foals was able to prevent clinical, clinicopathologic, and ultrasonographic abnormalities associated with PE in horses. As a second objective, humoral immune responses and fecal shedding of \textit{L. intracellularis} were compared between vaccinated and nonvaccinated foals.

**Materials and Methods**

**Animals**—Twelve healthy preweaned Quarter Horse foals between 4 and 5 months of age were included in the study. The foals belonged to the research herd at the University of California-Davis Center for Equine Health. The herd had no history of PE. Prior to study commencement, the foals were evaluated for any signs of illness by a veterinarian, who performed a full physical examination, CBC, and serum biochemical analysis. Blood (10 mL) was collected via jugular venipuncture into evacuated tubes with and without anticoagulant. All results were within reference intervals.

To ensure the foals had not previously been exposed to \textit{L. intracellularis}, whole blood was collected from all foals and their dams 7 days before the study began and tested for \textit{L. intracellularis}-specific antibody with an IPMA. All results were negative. Fecal samples were also collected during the same period and tested for \textit{L. intracellularis} DNA with a real-time PCR assay; and results confirmed the horses were not shedding the organism. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

**Experimental protocol**—The foals were randomly assigned to 3 groups. The vaccinated group consisted of 4 colts, and the nonvaccinated and control groups each consisted of 2 colts and 2 fillies. Foals in the vaccinated group received 30 mL of frozen-thawed \textit{L. intracellularis} vaccine intrarectally twice, 60 and 30 days prior to exposure to the organism (challenge). All efforts were made to administer the vaccine after defection to increase vaccine retention time within the rectum. The vaccine was handled and thawed in accordance with label instructions, and each vaccine dose was administered by use of a 12F, 20-cm urinary catheter. Vaccinated foals and their dams were kept separated from the remaining study foals to prevent environmental contamination and accidental ingestion of the \textit{L. intracellularis} vaccine strain, which can be shed in the feces for up to 12 days after first vaccine administration. \cite{12}

Five days before challenge, all mare and foal pairs were brought into the barn and kept in individual stalls (3.7 × 3.7 m). At that time, the foals received omeprazole paste (4 mg/kg, PO, q 24 h) for 5 consecutive days to increase stomach pH and potentially decrease the effect of low pH on the viability of the \textit{L. intracellularis} with which they would be challenged.

One day before the experimental challenge, foals were separated from their dams and kept in individual stalls (3.7 × 3.7 m) for the remainder of the study period (56 days). The foals had free choice of grass and alfalfa hay and water and received a commercial dietary product. \cite{8} Hay was provided on the ground twice daily, and the dietary product was provided in individual buckets once daily. Stalls were cleaned once daily. To prevent possible cross-contamination between foals, all people who interacted with them wore disposable gowns, shoe covers, and gloves. In addition, foot baths of diluted sodium hypochlorite (118 mL/4.5 L) solution were placed in front of each stall.

**Challenge inoculum**—The challenge isolate of \textit{L. intracellularis} originated from a foal that had undergone routine necropsy at the University of Minnesota College of Veterinary Medicine Veterinary Diagnostic Laboratory. On the basis of typical histopathologic and immunohistochemical findings, PE had been diagnosed. The molecular identity of the isolate was determined by multiple-locus variable number tandem repeat analysis. \cite{6} Enteric scrapings from the necropsied foal originally were grown in McCoy cells (mouse fibroblast cells) as described elsewhere, \cite{8} and the challenge organism was kept at −80°C until the study began. The same isolate has been used to establish a method by which to study PE in horses. \cite{15} Bacterial numbers were assessed by direct microscopic count after indirect immunoperoxidase staining by use of \textit{L. intracellularis}-specific antibody \cite{9} and by real-time PCR assay \cite{16}, and each challenge dose contained approximately 2.8 × 10^9 \textit{L. intracellularis} organisms.

On the challenge day, the frozen inoculum was thawed at 37°C and administered within 1 hour after complete thawing. Each of the vaccinated and nonvaccinated foals was sedated with detomidine hydrochloride \cite{16} (0.01 mg/kg, IV), and 50 mL of the inoculum was administered via nasogastric intubation. Control foals received 50 mL of minimal essential medium/nasogastric intubation.

**Monitoring and sample collection**—All foals were observed daily for general attitude and appetite. A complete physical examination was performed by veterinarians every day for the 56-day study period. Once weekly, the body weight of each foal was recorded to determine mean daily gain throughout the study period. Complete abdominal ultrasonography was performed by a veterinarian once weekly to assess intestinal wall thickness and motility and amount of free abdominal fluid. All examiners were blinded to each foal’s status.

Rectal swab specimens \cite{16} were collected from every foal every other day throughout the 56-day period for the quantitative molecular detection of \textit{L. intracellularis} as described elsewhere. \cite{17} Blood samples (approx 10 mL) were collected via jugular venipuncture into evacuated tubes \cite{16} at weekly intervals for serum total solids measurement and IPMA.
Proliferative enteropathy was diagnosed when a foal developed any of the main clinical signs of the disease (signs of depression, anorexia, rectal temperature > 38.6°C, peripheral edema, colic, changes in fecal consistency, and weight loss), hypoproteinemia (< 5.0 g/dL), or both as well as concurrent fecal shedding of L. intracellularis and detectable antibody against L. intracellularis. Foals were considered infected with L. intracellularis when they had positive results of PCR fecal testing after the first 7 days after inoculation and measurable antibody against the organism.

Foals that developed moderate to severe clinical signs (pyrexia, signs of depression, anorexia, colic, loose to watery fecal consistency, peripheral edema, and weight loss) or hypoproteinemia were treated with doxycycline hyclate (10 mg/kg, PO, q 12 h) for 10 days. Additional supportive treatment, including flunixin meglumine (1.1 mg/kg, IV, q 12 h) and replacement fluids (4 to 6 mL, IV, q 1 h), was given as needed.

Sample analysis—Rectal swab specimens were processed for nucleic acid purification within 2 hours after collection. Two milliliters of PBS solution was added to each specimen in a conical tube. Thereafter, each sample was mixed with a vortex device for 10 seconds and centrifuged at 13,000 × g for 10 seconds to remove fecal debris. Nucleic acid purification from 200 µL of supernatant fluid was performed by use of an automated nucleic acid extraction system in accordance with the manufacturer’s recommendations. The purified DNA was then analyzed with a real-time PCR assay for the presence of the aspA gene of L. intracellularis, as previously reported. Positive (DNA from cell-grown L. intracellularis) and negative (L. intracellularis-free DNA from fecal samples) control samples were used with each assay.

Absolute quantitation was performed through use of a standard curve for L. intracellularis and expressed as copy numbers of the aspA gene of L. intracellularis per swab specimen. The standard curve was determined by use of 10-fold dilutions of L. intracellularis derived from cell culture in McCoy cells added to L. intracellularis-free equine feces. A real-time PCR assay designed to identify a universal sequence of the bacterial 16S rRNA gene was used as a quality control (ie, efficiency of DNA purification and amplification) and as an indicator of fecal inhibition.

Serum was harvested from blood samples to determine the concentration of total solids by use of a refractometer as well as to measure the amount of IgG antibody against L. intracellularis via IPMA, as previously reported. All serum samples were screened at a dilution of 1:60, which is considered the standard cutoff titer for L. intracellularis testing via IPMA. Positive serum samples (titer 60) were tested to endpoint dilution, and titers were reported as the reciprocal of the dilution.

Statistical analysis—Statistical analysis was performed by use of the Wilcoxon-Mann-Whitney test to assess differences in daily weight gain and serum total solids concentration among the groups. Differences in fecal shedding (amount and duration) and titers of antibody against L. intracellularis were calculated by use of the AUC of time versus the number of aspA gene copies per swab specimen and AUC of time versus IPMA titers against L. intracellularis for each of the foals. The Wilcoxon-Mann-Whitney test was used to determine whether differences were significant (P < 0.05).

Results

Animals—No adverse reactions attributed to the vaccine or administration procedures were evident among any foals in the vaccinated group. Clinical signs compatible with PE were observed only in the nonvaccinated group of foals. Three such foals developed moderate to severe clinical signs of PE, including signs of depression, anorexia, diarrhea ranging from soft and semiformal (ie, cow-pie consistency) to watery, colic, and peripheral edema. Two foals developed moderate clinical signs between days 21 and 24 after challenge and were treated with doxycycline for 10 days. One foal had a sudden onset of PE and developed signs of depression, complete anorexia, and colic on day 19 after challenge. It did not respond to antimicrobial and supportive treatment and was euthanized 5 days later. A full necropsy of that foal revealed diffusely gas- and fluid-filled small and large intestines with thickened wall of the ileum. Histologic evaluation showed diffuse and severe PE, with large numbers of intracellular organisms identified through Lawsonia-specific immunohistochemical analysis. The fourth nonvaccinated foal remained unaffected throughout the entire study period.

Figure 1—Mean ± SD daily weight gain in foals intrarectally vaccinated with a porcine Lawsonia intracellularis vaccine (white bars; n = 4) and nonvaccinated foals (light gray bars; 4) that were inoculated with an equine strain of L. intracellularis and un inoculated, nonvaccinated control foals (dark gray bars; 4). Value for the nonvaccinated group differs significantly (P < 0.05) from that of the vaccinated group. Value for the control group differs significantly from that of the nonvaccinated group. Value for the control group differs significantly from that of the vaccinated group.
No differences in body weight were evident at study commencement among the foal groups. However, daily weight gain differed significantly throughout the entire study period between vaccinated and nonvaccinated foals ($P = 0.02$) and between nonvaccinated and control foals ($P = 0.02$; Figure 1). Daily weight gain by study week was only significantly different between vaccinated and control foals ($P = 0.01$) during the third week. Nonvaccinated foals maintained a significantly lower daily weight gain, compared with that of vaccinated foals for weeks 2 through 5 and compared with that of control foals for weeks 3 to 5.

Hypoproteinemia (< 5.0 g/dL) was recorded for 3 of 4 nonvaccinated group foals, with the lowest values ranging from 4.1 to 4.7 g/dL between days 21 and 28 after challenge. For the 2 surviving nonvaccinated foals with clinical signs, hypoproteinemia resolved within 28 days following the onset of hypoproteinemia. Significant differences in serum total solid concentrations were detected between vaccinated and nonvaccinated foals on days 28 and 35 after challenge (Figure 2). All vaccinated and control foals maintained serum total solid concentrations within reference limits (5.5 to 6.6 g/dL).

Ultrasonographic abnormalities were observed in 3 of 4 nonvaccinated foals on days 21 (n = 3 foals) and 28 (2) after challenge and consisted of mild increases in the volume of abdominal free fluid and moderately thickened small intestinal loops, with wall thicknesses ranging from 4.4 to 6.0 mm (reference limit, ≤ 3 mm). No ultrasonographic abnormalities were observed in the vaccinated and control foals.

Molecular testing—*Lawsonia intracellularis* DNA was successfully extracted from all rectal swab specimens, as indicated by positive PCR signals for the universal bacterial 16S rRNA gene, ruling out inadequate nucleic acid purification and inadequate amplification efficiency. Fecal shedding of *L* intracellularis was detected via the PCR assay in 2 of 4 vaccinated foals and in all nonvaccinated foals. All control foals had negative PCR assay results for *L* intracellularis in rectal swab specimens for the entire study period. Detection of *L* intracellularis started on day 12 for the 2 vaccinated foals and between days 10 and 14 after challenge for the nonvaccinated foals (Figure 3). Fecal shedding lasted for 7 and 3 days for 2 vaccinated foals. The 3 surviving nonvaccinated foals shed *L* intracellularis in feces for 17 to 27 days, and the nonsurviving nonvaccinated foal shed *L* intracellularis in feces for 9 days.

The mean AUCs for time versus the number of *L* intracellularis aspA gene copies per specimen were significantly different between vaccinated and nonvaccinated foals. The multiple-locus variable number tandem repeat profile of *L* intracellularis from the feces of all foals with positive PCR assay results and from intestinal scrapings from 1 foal was identical to the profile of the original challenge organism.
Evidence to support this conclusion consisted of a lack with a virulent isolate of equine origin. L intracellularis ling foals vaccinated intrarectally with an avirulent live nonvaccinated foals. The mean AUCs for time versus IPMA titers against after challenge, respectively, and maintained high titers 3 nonvaccinated foals seroconverted by day 14 and 21 intracellularis from days 28 to 56 after challenge. One and 14 after challenge administration (Figure 4). All vaccinated foals maintained high antibody titers against L intracellularis for the remaining study period. The mean AUCs for time versus IPMA titers against L intracellularis were similar between vaccinated and nonvaccinated foals.

Discussion

Findings of the present study suggested that weanling foals vaccinated intrarectally with an avirulent live frozen-thawed porcine vaccine against L intracellularis were protected against clinical PE following exposure with a virulent L intracellularis isolate of equine origin. Evidence to support this conclusion consisted of a lack of clinical disease, absence of hypoproteinemia and ultrasonographic abnormalities compatible with PE, and significant reduction in the fecal shedding of L intracellularis in vaccinated versus nonvaccinated foals.

Despite use of the same L intracellularis isolate and a similar challenge dose as in another study, the clinical signs observed in 3 of 4 nonvaccinated study foals were more severe than the mild and self-limiting signs reported for that other study. Abnormal clinical signs were first observed in the nonvaccinated foals between days 19 and 24 after challenge, which is similar to previous findings. However, clinical signs in foals developed later than in swine, which can have changes in fecal consistency as early as 4 to 7 days after challenge. Overall, it appears that weaning foals are slower to develop proliferative pathological changes and hence clinical and clinicopathologic changes than are pigs. Such differences between species may be attributable to age at time of exposure, degree of physiologic stress, strain and dose of L intracellularis challenge, and rate of pathological change.

Of interest in the present study was the development of variable clinical signs among the nonvaccinated foals, with a severe onset of gastrointestinal disturbance in 1 foal, a more progressive development of signs in 2 foals, and mild clinical signs in the fourth foal. Variations in clinical signs have been established in pigs, in which 3 manifestations have been characterized: chronic, acute, and subclinical. The chronic form is the most prevalent and is characterized by diarrhea, rough coat, decreased feed consumption, and reduced weight gain. The acute form usually affects slightly older pigs and is characterized by severe intestinal hemorrhage, pyrexia, and sudden death.

In subclinically infected pigs, poor growth is the only sign. Subclinical disease in experimentally infected foals has been reported and was evident solely as a decrease in daily weight gain, compared with that in control foals. Although the financial impact of a low rate of weight gain has been established for growing pigs, it needs to be determined whether such a condition has a short-term or long-term impact in weanling foals. Results of a study involving swine suggest that vaccination against L intracellularis may not only prevent PE but may also increase resistance and tolerance against other infectious diseases and management losses. Longitudinal studies are needed to investigate the long-term effect of subclinical disease on future performance and health of affected foals.

In foals with PE, hypoproteinemia due to hypoalbuminemia is believed to develop as a combined effect of feed intake decrease, malabsorption, and protein-losing enteropathy. Hypoproteinemia is a consistent hallmark of PE in horses and has generally not been observed in or reported for other species with PE caused by L intracellularis infection. Hypoproteinemia was evident within a few days after the development of clinical PE in 3 nonvaccinated foals in the present study and was detected concurrently with the development of thickened small intestinal loops. The hypoproteinemia resolved in the 2 nonvaccinated survivors within 28 days after antimicrobial treatment began. This interval is shorter than in previous reports and likely reflects the interval to initial diagnosis of PE and differences in severity of initial hypoproteinemia, therapeutic management, and interval to resolution of pathological abnormalities.

Fecal shedding of L intracellularis was significantly lower in vaccinated versus nonvaccinated foals, which is in agreement with shedding in a study of the vaccine’s efficacy in pigs. Onset of fecal shedding was similar between the 2 vaccinated and the 4 nonvaccinated foals and similar to that in another foal study. As previously reported, it appears that the magnitude and duration of fecal shedding was directly correlated with the clinical severity of PE. Fecal shedding was not only influenced by the vaccine status but also by doxycycline treatment in 2 nonvaccinated foals. These 2 foals had a rapid decline in pathogen loads, as indicated by lack of L intracellularis detection in feces between 4 and 6 days after treatment initiation.

![Figure 4—Serum IgG antibody titers against L intracellularis in the individual vaccinated (circles) and nonvaccinated (squares) foals in Figure 1. No measurable response against L intracellularis was evident in the serum of the 4 control foals at any point. Notice that the y-axis is not to scale. Days –60 and –30 represent the days the first and second vaccines, respectively, were administered in vaccinated foals.](image-url)
A high degree of L intracellularis shedding in feces represents an increase in the potential for environmental contamination and exposure to susceptible animals. Information regarding the survival of L intracellularis in the environment is lacking, mainly owing to the difficulty experienced when attempting to grow the bacteria directly from fecal matter. Fecal-oral transmission has been reported between an experimentally infected foal and a naïve sentinel herdmate.15 Results reported here highlight the need to undertake proper biosecurity measures when hospitalizing affected foals and the potential of the L intracellularis vaccine to reduce environmental contamination by preventing or minimizing fecal shedding in vaccinated foals. Our study is the first to show the effect of treatment on experimentally infected horses and their fecal shedding, and the results correspond with those in a clinical report.16

Serologic responses were detected in all vaccinated and nonvaccinated study foals following challenge administration. The lack of detectable seroconversion following vaccine administration was not unexpected and was in agreement with findings in pigs.8,9 Absence of a detectable antibody response might have been attributable to the vaccine type (ie, lyophilized vs frozen-thawed), route of vaccine administration, and amount of vaccine administered as well as the serologic method used to detect specific antibody against L intracellularis. Collectively, swine studies8,9 have shown that seroconversion is not necessary to achieve protection and that nonhumoral factors simulated by the avirulent L intracellularis vaccine strain likely play an important role in immunity. Immunologic responses against L intracellularis during infection have remained largely uncharacterized. Secretory IgA and a cell-mediated immune response likely play a role in overcoming natural infection with L intracellularis.25,26

References