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Short communication

Temporal detection of *Lawsonia intracellularis* using serology and real-time PCR in Thoroughbred horses residing on a farm endemic for equine proliferative enteropathy

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ABSTRACT

The goals of this study were to evaluate titers of antibodies against *Lawsonia intracellularis* in 68 resident broodmares from a farm known to be endemic for equine proliferative enteropathy (EPE) and to evaluate maternal antibodies, occurrence of seroconversion and fecal shedding in their foals. Serum samples collected from mares at delivery and from foals pre- and post-colostrum ingestion and monthly thereafter were tested for the presence of *L. intracellularis* antibodies by immunoperoxidase monolayer assay (IPMA). Further, feces collected from mares at delivery and foals post-partum and monthly thereafter were assayed for *L. intracellularis* using real-time PCR. Thirty-seven mares (54.4%) had detectable antibody titers ($\geq$60) against *L. intracellularis* by IPMA at the time of foaling. Passive transfer of colostral antibodies against *L. intracellularis* was documented in 37 foals (54.4%) and the colostral antibodies remained detectable in the serum of foals for 1–3 months. Overall, 22 foals (33.3%) showed evidence of natural exposure to *L. intracellularis* throughout the study period, however, none of the study foals developed signs compatible with EPE. The serological results showed that mares residing on a farm known to be endemic for EPE are routinely exposed to *L. intracellularis* and that antibodies against *L. intracellularis* are passively transferred to foals.

1. Introduction

Equine proliferative enteropathy (EPE) is an emerging enteric disease of foals caused by the obligate intracellular organism *Lawsonia intracellularis* (Lawson and Gebhart, 2000). The disease has been reported from North America, Europe, Australia, and South Africa. Affected foals, generally 4–7 months of age, display lethargy, weight loss, peripheral edema, diarrhea and colic. An antemortem diagnosis of EPE is based on clinical signs, the presence of hypoproteinemia, thickening of segments of the small intestinal wall observed on abdominal ultrasonography, positive serology and molecular detection of *L. intracellularis* in feces (Lavoie and Drolet, 2007). Transmission of *L. intracellularis* is thought to occur through the ingestion of feed or water contaminated with *L. intracellularis*-infected feces from free-living or domestic animals (Lawson and Gebhart, 2000; Lavoie and Drolet, 2007; Pusterla et al., 2008). Although the clinical entity, diagnostic work-up and treatment of EPE are well established and described, the epidemiological aspects of the disease within a herd have remained poorly investigated. The goals of this study were to evaluate titers of antibodies against *L. intracellularis* in broodmares from a farm known to be endemic for EPE, and

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to evaluate maternal antibodies, occurrence of seroconversion and fecal shedding in foals.

2. Materials and methods

2.1. Study farm and population

For the purpose of the study, the authors selected one farm located in southern California with endemic occurrence of EPE. Historically, this farm has had an increasing number of yearly cases of EPE with a culmination of 6 cases, including two fatalities, in 2006. The Thoroughbred breeding farm keeps approximately 300 horses on 120 acres. During the 2007 foaling season, all of the 68 resident broodmares and their foals were enrolled in the study and samples were collected from January to December. Although defining risk factors for exposure to *L. intracellularis* was not part of this study, the management practices included raising the foals on irrigated pastures and supplementing hay and grain in elevated feeders. Broodmares were kept on irrigated pastures and supplemented with hay and grain fed on the ground during the last trimester of gestation. Mares and foals had no direct or indirect exposure to manure from domestic or wild pigs. A variety of free-living animals, including opossums, raccoons, coyotes, skunks, ground squirrels, rodents, rabbits, bats and birds were present on the premise.

Serum was collected from mares following parturition, from foals pre- and post-colostrum ingestion to document passive transfer of colostral antibodies and from foals on a monthly basis thereafter. Additionally, feces from mares at delivery and foals post-partum and monthly thereafter were collected for the molecular detection of *L. intracellularis*.

2.2. Total solid concentration, serology and PCR analysis

The serum collected from the foals was used to determine the concentration of total solids using a refractometer and to measure anti-*Lawsonia intracellularis* specific IgG antibodies by immunoperoxidase monolayer assay (IPMA) as previously reported (Guedes et al., 2002a). Feces were processed for nucleic acid purification within 24 h of collection. Two milliliters of PBS were added to 2 g of feces in a conical tube. Thereafter, each sample was vortexed for 10 s and centrifuged at 13,000 × *g* for 10 s. Nucleic acid purification from 180 μl of supernatant fluid was performed using an automated nucleic acid extraction system (CAS-1820 X-tractor Gene, Corbett Life Science, Sydney, Australia) according to the manufacturer's recommendations. The purified DNA was then analyzed by real-time PCR for the presence of the *aspartate ammonia lyase* gene of *L. intracellularis* as previously reported (Pusterla et al., 2008). Positive (DNA from cell culture-grown *L. intracellularis*) and negative (*L. intracellularis*-free DNA from fecal samples) DNA controls were used with each run.

Statistical data analysis was performed using Wilcoxon–Mann–Whitney tests to look for differences in titers between mares and foals.

3. Results

Thirty-seven mares (54.4%) had positive serum titers (≥60) against *L. intracellularis* by IPMA at the time of foaling with titers ranging from 60 to 240 (14 mares with a titer of 60, 12 mares with a titer of 120 and 11 mares with a titer of 240; mean titer of 133). The seroprevalence in mares increased with each foaling month (3/9 (33%) mares in January, 10/21 (48%) mares in February, 11/21 (52%) mares in March, 10/14 (71%) mares in April, 3/3 (100%) mares in May). None of the fecal samples collected from all mares at foaling time tested PCR positive for *L. intracellularis*.

All foals had negative antibody titers (<60) against *L. intracellularis* prior to colostrum ingestion. None of the foals had evidence of failure of passive transfer based on adequate IgG levels (≥800 mg/dl) using a commercial IgG test (SNAP Foal IgG Test, IDEXX Laboratories, Westbrook, ME, USA). Passive transfer of colostral antibodies against *L. intracellularis* was documented in 37 foals (54.4%) with titers ranging from 60 to 240 (17 foals with a titer of 60, 12 foals with a titer of 120 and 8 foals with a titer of 240; mean titer of 118). Twenty-five seropositive foals were born from seropositive mares and 12 seropositive foals were born from mares with undetectable antibodies against *L. intracellularis*. An additional 11 seronegative foals were born from seropositive mares and 20 mare and foal pairs had no detectable antibodies against *L. intracellularis* (Table 1). When the 68 mare and foal serum titers against *L. intracellularis* were compared against each other, 34 pairs had similar titers, 21 foals had lower serum titers than their corresponding mares and 13 foals had higher serum titers than their mares. Titers from seropositive foals born from seropositive mares (mean titer of 156) were significantly higher ($p = 0.0367$) than titers from seropositive foals born from seronegative mares (mean titer of 93). Colostral antibodies remained detectable in the serum of foals for 1 (32 foals), 2 (4) and 3 (1) months. All fecal samples collected from foals at 24 h of age tested PCR negative for *L. intracellularis*.

Overall, 22 foals (32.3%) showed evidence of natural exposure to *L. intracellularis* throughout the study period. The titers in these foals ranged from 60 to 240 (15 foals with titers of 60, 6 foals with titers of 120, one foal with a titer of 240; mean titer of 84). The age of onset of seroconversion varied from 2 to 7 months (mean age 3.6 months, median age 3 months; Table 2). Thirteen of the 22 foals had documented prior passive transfer of colostral antibodies against *L. intracellularis*, while the additional 9

Table 1
Results of IPMA against *Lawsonia intracellularis* for serum IgG in resident mares and their foals 24 h post-colostrum ingestion.

	Mare titer			
	≤60	60	120	240
Foal titer				
≤60	20	9	1	1
60	6	3	5	3
120	4	1	5	2
240	2	0	0	6

Table 2
Age of onset of seroconversion to *Lawsonia intracellularis* in 68 resident foals.

Age (months)	Number seropositive foals (titer ≥ 60)	Number seronegative foals (titer < 60)	Mean titer for seropositive foals
2	8	60	75
3	4	64	60
4	3	64	120
5	4	63	90
6	2	66	90
7	1	67	240

foals had undetectable antibodies against *L. intracellularis* following colostrum ingestion. The measurable antibodies remained detectable for 1 month in 20 foals and 2 months in 2 foals.

None of the 68 study foals developed signs compatible with EPE during the entire study period and the concentration of total solids in the serum of all foals remained within the normal reference range (5.8–8.7 g/dl). Only one single fecal sample collected from a 3-month-old foal tested PCR positive for *L. intracellularis*. This foal remained healthy and became seropositive (titer of 60) on the following collection time.

4. Discussion

The study has shown that more than half of the resident broodmares tested seropositive at the time of foaling. The results are in agreement with previous studies showing that adult horses on endemic farms are commonly exposed to *L. intracellularis* (Feary et al., 2007). Although it was not the scope of this study to follow the mares serologically on a monthly basis, it appeared that seroprevalence varied by foaling month. This may indirectly reflect the exposure rate of these horses in the environment. Previous studies have determined that a variety of wild animals are shedding *L. intracellularis* via feces on endemic swine and equine farms (Friedman et al., 2008; Pusterla et al., 2008). Management practices, such as feeding hay and grain on the ground, may have contributed to the high seroprevalence rate in broodmares by allowing potential reservoir hosts to contaminate feed. Healthy resident mares did not shed *L. intracellularis* in feces at the time of foaling. This is in agreement with studies performed in pigs showing that fecal samples collected from seropositive and seronegative gilts close to farrowing are negative for *L. intracellularis* (Guedes et al., 2002b).

This study is the first one to document passive transfer of colostal antibodies against *L. intracellularis* from mares to foals. The difference in titers between seropositive foals born from seropositive or seronegative mares reflects the concentration of specific antibodies in the colostrum and the ingestion or absorption of different amounts of colostrum. Passive transfer of maternally derived antibodies against *L. intracellularis* has been previously reported in pigs (Holyoake et al., 1994; Wendt et al., 2000; Guedes et al., 2002b). Collectively, these studies showed that piglets born from seropositive sows had low antibody titers against *L. intracellularis* that usually lasted for only 3 weeks. Similar results were found in the study foals with measurable colostral antibodies against *L. intracellularis* been detectable for less than 1 month in 86.5% of the seropositive foals.

The results reflect a high exposure rate to *L. intracellularis* during the pre-weaning period, since 54% of seropositive foals seroconverted during the first 3 months of life. The low and short-lived immunological response following natural infection with *L. intracellularis* in the study foals is similar to previous studies in pigs and horses (Lavoie et al., 2000; Guedes et al., 2002b; Feary et al., 2007).

Based on the rare detection of *L. intracellularis* in the feces of the study animals, it does not appear that healthy foals are actively contributing to the number of organisms shed into the environment. This is in sharp contrast to piglets, shown to shed *L. intracellularis* early in life and for long duration; thus, contributing to the build up of bacteria in the environment (Lopez et al., 2000; Guedes et al., 2002b).

It is unclear to the authors why no clinical cases of EPE were diagnosed during the study period. Differences in exposure rates may have accounted for the lack of clinical cases. This is exemplified by the higher seroprevalence of 29.7% measured in October of 2006 in the resident foal population (data not shown), compared to 1.5% measured in October of 2007. It remains to be determined if age of onset of exposure and presence of maternally derived antibodies against *L. intracellularis* are associated with decreased infection rate in foals.

In conclusion, the present study showed that colostral antibodies against *L. intracellularis* were passively transferred to foals born on a farm endemic for EPE. Further, the seroprevalence against *L. intracellularis* in mares and foals varied with the foaling months and age, respectively. Despite high exposure to *L. intracellularis* in foals, no clinical EPE cases were recorded.

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