Epidemiological survey on farms with documented occurrence of equine proliferative enteropathy due to *Lawsonia intracellularis*

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PROLIFERATIVE enteropathy caused by the obligate intracellular organism *Lawsonia intracellularis* has been described in a number of domestic and wild animal species (Lawson and Gebhart 2000). In horses, the disease is known as equine proliferative enteropathy (EPE) and has been reported from North America (most American states and Canada), Europe (Great Britain, Belgium and Switzerland) and Australia (Lavoie and Drolet 2007). EPE commonly affects weanling foals at four to seven months of age and has a sporadic occurrence, although outbreaks on breeding farms have been reported (Lavoie and others 2000). Affected weanlings commonly show rapid weight loss, lethargy, depression, fever, subcutaneous oedema due to hypoproteinaemia, diarrhoea and colic. The epidemiology of EPE has remained poorly investigated and the transmission of infection in foals may occur through the ingestion of feed or water contaminated with *L intracellularis*-infected faeces from free-living or domestic animals (Lawson and Gebhart 2000, Lavoie and Drolet 2007). The objectives of this study were to determine the seroprevalence of *L intracellularis* in resident foals from two farms with documented cases of EPE, and to determine if subclinical foals contribute to the shedding of *L intracellularis*.

The study was performed on two farms in California. The farms were selected based on one or more documented clinical case of EPE. All documented cases had clinical and clinicopathological findings compatible with EPE, a positive antibody titre to *L intracellularis* by immunoperoxidase monolayer antigen assay (IPMA) and positive faeces for *L intracellularis* by real-time PCR. Farm 1, a Percheron breeding farm with 190 resident horses housed on 20 acres, was visited one week following the diagnosis of EPE in a six-month-old filly (in December 2005). Farm 2, a thoroughbred breeding farm with 300 resident horses housed on 40 acres, was visited twice (in October and December 2006) following the diagnosis of five weanlings with EPE. The first visit on farm 2 occurred six weeks after the last of the five confirmed cases of EPE. During each visit, a physical examination was performed and blood and faecal samples were collected from each resident foal.

The serum collected from the foals was used to determine the concentration of total solids using a refractometer, and to measure *L intracellularis*-specific antibodies by IPMA as reported by Guedes and others (2002). Faeces were processed for nucleic acid purification within 48 hours of collection. Phosphate-buffered saline (2 ml) was added to 2 g faeces in a conical tube. Each sample was vortexed for 10 seconds and centrifuged at 13,000 *g* for two minutes. Nucleic acid purification from 180 µl supernatant fluid was performed using an automated nucleic acid extraction system (CAS-1820 X-tractor Gene; Corbett Life Science) according to the manufacturer's recommendations. The purified DNA was then analysed by real-time PCR using a previously validated assay targeting the 16S rRNA gene of *L intracellularis* (Feary and others 2007). Positive (DNA from cell-grown *L intracellularis*) and negative (*L intracellularis*-free DNA from faecal samples) DNA controls were used with each run.

A total of 11 and 91 resident foals were sampled on farms 1 and 2, respectively. Sixty-five of the 91 foals from farm 2 were sampled a second time two months after the first collection. The previously diagnosed foals with EPE (one on farm 1 and five on farm 2) were among the sampled foals. The age of the resident foals at the first sampling ranged from 5·5 to 8·4 months (mean [sd] 7·1 [1·1] months) and from 4·7 to 9·3 months (7·6 [1·3] months), for farms 1 and 2, respectively. The population of foals comprised six colts and five fillies on farm 1, and 55 colts and 36 fillies on farm 2. On the day of sampling, all foals appeared in good health and none was showing clinical signs compatible with EPE.

With the exception of the index case from farm 1 (56 g/l), all remaining foals from the same farm had solid serum concentrations in the reference range of 58 to 87 g/l, ranging from 58 to 67 g/l (mean [sd] 61 [4] g/l). The concentration of total solids of all resident foals from farm 2 was within reference limits and ranged from 58 to 72 g/l (mean [sd] 65 [3] g/l) in October and from 58 to 69 g/l (mean [sd] 66 [3] g/l) in December. Five of the 11 foals (45·5 per cent) from farm 1 had a positive titre (≥30) to *L intracellularis* by IPMA (Table 1). All seropositive foals, including the index case, had titres of 120.

The serological data for farm 2 showed that 27 of 91 foals (29·7 per cent) and 22 of 65 foals (33·8 per cent) had a positive titre to *L intracellularis* in October and December, respectively (Table 1). The 27 seropositive foals from farm 2 that were tested in October had titres ranging from 30 to 1920 (Table 2), while the titres ranged from 30 to 240 for the 22 seropositive foals tested in December. The highest measured titre of 1920 belonged to a healthy eight-month-old thoroughbred colt with no prior history or signs of EPE. The titre of this foal decreased to 240 in December. From the 65 foals from farm 2 with dual serological results, 38 foals were seronegative (<30) for both sampling times, five foals became seronegative during the two-month period, six foals became seropositive, four foals kept the same titre, eight foals had a two-fold decrease in titre, two foals had a four-fold decrease in titre, one foal had an eight-fold decrease in titre, and one foal had a two-fold increase in titre (Table 2). The five previously diagnosed foals with EPE from farm 2 had titres ranging from 240 to 480 in October (three titres of 240 and two titres of 480). With the exception of one foal that kept the same

TABLE 1: Total solid concentrations, serological results by immunoperoxidase monolayer antigen assay and molecular detection of *Lawsonia intracellularis* by real-time PCR from resident foals from two farms with documented cases of equine proliferative enteropathy

Farm	Collection time	Total solid concentration (g/l)	*L intracellularis* serology positive/tested (%)	Titre range (mean titre for positive)	PCR results positive/tested
1	December 2005	56-67	5/11 (45·5)	120 (120)	0/11
2	October 2006	58-72	27/91 (29·7)	30-1920 (295)	0/91
	December 2006	58-69	22/65 (33·8)	30-240 (146)	0/65

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TABLE 2: Serological results by immunoperoxidase monolayer antigen assay against *Lawsonia intracellularis* of 27 foals from farm 2

Foal number	Titre		Interpretation
	October 2006	December 2006	
1	240	120	Two-fold decrease
2	240	120	Two-fold decrease
3	120	<30	Seroreversion
4	480	120	Four-fold decrease
5	240	240	Identical titre
6	240	<30	Seroreversion
7	480	240	Two-fold decrease
8*	240	120	Two-fold decrease
9	<30	60	Seroconversion
10	<30	120	Seroconversion
11	240	240	Identical titre
12*	240	240	Identical titre
13	120	60	Two-fold decrease
14	<30	120	Seroconversion
15	<30	120	Seroconversion
16	60	<30	Seroreversion
17	30	60	Two-fold increase
18*	480	240	Two-fold decrease
19*	480	240	Two-fold decrease
20*	240	120	Two-fold decrease
21	<30	120	Seroconversion
22	120	120	Identical titre
23	480	120	Four-fold decrease
24	1920	240	Eight-fold decrease
25	<30	30	Seroconversion
26	60	<30	Seroreversion
27	30	<30	Seroreversion

* Foals with documented equine proliferative enteropathy

titre of 240, the remaining four index cases had a two-fold lower titre in December (two titres of 120 and two titres of 240). All faecal samples tested negative by PCR for the presence of *L intracellularis*.

The case definition of EPE in an affected weanling foals includes clinical signs suggestive of an enteric infection (anorexia, depression, fever, peripheral oedema, colic and diarrhoea), the presence of hypoproteinaemia, a thickening of segments of the small intestinal wall observed on abdominal ultrasonography, positive serology and molecular detection of *L intracellularis*. Confirmation of the diagnosis by the presence of characteristic histopathological changes and the detection of intracellular bacteria within the apical cytoplasm of proliferating crypt epithelial cells by means of special staining or immunohistochemistry, is rarely warranted since most of the affected foals respond favourably to specific antimicrobial treatment and supportive care. The case definition was applied in the study cases in order to select the two farms. For the purpose of the study, the authors selected one farm with sporadic occurrence of the disease (farm 1) and one farm with enzootic occurrence (farm 2). While farm 1 has had no additional confirmed cases of EPE since 2005, farm 2 has had multiple confirmed cases of EPE on an annual basis. Although defining risk factors for the development of EPE was not part of this study, the two farms shared a few epidemiological similarities such as raising their foal crop on irrigated pastures, no direct or indirect exposure to manure from domestic or wild pigs, and the presence of a variety of free-living animals such as opossums, raccoons, coyotes, skunks, cats, squirrels, bats and birds on the premises.

Despite the high seroprevalence of *L intracellularis* in the foals from the two study farms, the incidence of clinical EPE cases remained low: one of 11 (9·1 per cent) foals for farm 1 and five of 91 (5·5 per cent) foals for farm 2. This raises questions about the susceptibility of individual animals, as well as risk factors associated with clinical disease. As observed in pigs, factors that may predispose susceptible foals to developing EPE are weaning, transportation, overcrowding, decreasing specific colostral antibodies, diet changes and concurrent diseases (parasitism and pneumonia) (Lavoie and Drolet 2007). The potential role of subclinically infected horses in the transmission of *L intracellularis* to susceptible foals remains to be determined. However, based on the study results, it does not appear that healthy foals are shedding detectable *L intracellularis* in faeces. This may be in sharp contrast to foals affected by EPE, since such foals may excrete the bacteria for an extended period of time if left untreated (Lavoie and Drolet 2007). The authors cannot rule out that the lack of detectable *L intracellularis* in the study foals may have been associated with the detection limit of the molecular assay, the intermittent shedding of the pathogen or the collection times. Several PCR assays have been established and used to document *L intracellularis* shedding in pigs and other animals, with a reported analytical sensitivity ranging from 1 to 1000 target molecules per reaction tube (Jones and others 1993, Lindecrona and others 2002, Herbst and others 2003, Jacobson and others 2004). The real-time PCR assay used in the present study has an analytical sensitivity of 40 target molecules per reaction tube.

The seroprevalence to *L intracellularis* in the study foals was high for both farms, implying high bacterial exposure. Reports focusing on the exposure rate of healthy horses from farms with documented EPE cases are rare. Two studies have been unable to document the presence of measurable *L intracellularis*-specific antibodies in healthy adult horses and foals housed with index cases (Lavoie and others 2000, Dauvillier and others 2006). On the other hand, *L intracellularis*-specific antibodies were reported from the dam of one affected filly (Schumacher and others 2000), as well as from 12 of 16 serum samples collected from healthy adult horses and weanlings housed with an index case (Feary and others 2007). The present study represents the first serological study aimed at determining the prevalence of *L intracellularis* in large populations of susceptible foals. The serological study data most likely represent peak prevalence for both farms, since the serum samples were collected following the last clinical EPE case on each farm. Future temporal and spatial studies will be necessary to determine the exact time point and location of exposure by resident foals. It appears that, on farm 2, for which two sets of serum samples were available from 65 foals, reduced exposure to *L intracellularis* must have occurred in the two months following the first collection time, based on decreasing titres in the majority of the tested animals.

The high seroprevalence in the resident foal population also highlights the importance of not relying solely on serology to achieve a definitive diagnosis of EPE in a suspected case. The results are in sharp contrast to the general belief that exposure to *L intracellularis* in the absence of disease will not elicit a detectable antibody response. Several reported cases of EPE have documented the absence of *L intracellularis*-specific antibodies at the onset of disease (Dauvillier and others 2006, Sampieri and others 2006, Feary and others 2007). Immunological responses against *L intracellularis* during infection have remained mostly uncharacterised and appear to be associated with moderate responses (Smith and Lawson 2001). Interestingly, the serological results from infected and diseased resident foals from farm 2 were not statistically different when compared (Mann-Whitney test, $P>0{\cdot}05$).

In conclusion, the present study documents the high seroprevalence of *L intracellularis* in resident foals from farms with documented cases of EPE. Furthermore, the study results show that subclinical cases of EPE, determined by a low serum total solid concentration and faecal shedding of *L intracellularis*, are uncommon.

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