Effects of administration of an avirulent live vaccine of *Lawsonia intracellularis* on mares and foals

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Lawsonia intracellularis, an obligate intracellular organism, is the aetiological agent of proliferative enteropathy in a number of domestic and wild animal species (Lawson and Gebhart 2000). In horses, equine proliferative enteropathy (EPE) is considered an emerging disease and has been reported with increasing frequency in North America, Europe, Australia and more recently South Africa (Lavoie and Drolet 2007). EPE commonly affects weanling foals, from four to seven months of age, causing lethargy, weight loss, peripheral oedema, fever, colic and diarrhoea (Lavoie and others 2000). Diagnosis relies mainly on the clinical signs, the presence of hypoproteinaemia, thickening of segments of the small intestinal wall observed on abdominal ultrasonography, positive serology and molecular detection of *L intracellularis* in faeces (Lavoie and Drolet 2007). The epidemiology of EPE is poorly understood and no specific measures have been established for preventing the disease. Prevention strategy has been best described in pigs using antimicrobials in feed and a commercially available *L intracellularis* modified-live vaccine (Lawson and Gebhart 2000, Kroll and others 2004, McOrist and Smits 2007). Recent work has shown that a detectable humoral response can be measured in foals administered the modified-live *L intracellularis* vaccine (Pusterla and others 2008b). This short communication describes a study to investigate the humoral immune response and onset and duration of faecal shedding in healthy pregnant broodmares, and to determine if specific maternal antibodies against *L intracellularis* were passively transferred to foals.

Twelve healthy, pregnant quarter horse mares aged between four and 19 years old used for the study. The mares belonged to the research herd at the Center for Equine Health, at the University of California, Davis. The herd had no history or recorded cases of EPE. All the mares were examined for signs of ill health and a complete blood cell count was performed at the beginning of the study. All results were within reference ranges. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

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The 12 pregnant mares were divided into one group of eight animals (vaccinated, group 1) and one group of four animals (unvaccinated sentinels, group 2). The mares were kept in small groups on dirt paddocks until close to foaling. The mares had free choice of grass, alfalfa hay and water, and were supplemented daily with a commercial mare and foal supplement. Following foaling, mare and foal pairs were kept in stalls and moved back to dirt paddocks four to six weeks post-foaling. The immunisation protocol was extrapolated from a recent study on foals showing that the strongest humoral immune response to *L intracellularis* occurred following intrarectal administration of the modified-live *L intracellularis* vaccine strain (Pusterla and others 2008b). After evacuation of the rectum, each of the eight mares in group 1 received 50 ml of the *L intracellularis* vaccine (Enterisol Ileitis; Boehringer Ingelheim Vetmedica) intrarectally, dispensed via a 12 F 20 cm urinary catheter. The frozen vaccine was handled and thawed in accordance with label instructions. The mares were immunised three to five weeks before the expected foaling dates. The four unvaccinated mares in group 2 served as unvaccinated sentinels and were housed with the vaccinated mares. Before the beginning of the study, serum samples were collected from all mares and tested for *L intracellularis* antibodies by immunoperoxidase monolayer assay (IPMA) (Guedes and others 2002b), in order to document a seronegative status in each animal. In addition, faecal samples were collected and tested for *L intracellularis* DNA by real-time PCR, in order to document the absence of faecal shedding before the start of the study.

All mares were observed daily for general attitude and appetite. A complete physical examination was performed once a week for the entire study period (12 weeks). Serum was drawn from each mare once a week for 12 weeks following vaccination, and rectal swabs were collected using rayon tipped applicators (Puritan Medical Products) every other day for a period of four weeks after vaccination.

Following parturition, colostrum from the dam and serum from the foal before and after colostrum ingestion were collected to determine antibody titres against *L intracellularis* by IPMA. Thereafter, serum was collected from all foals once a week for eight weeks for serological analysis.

The serum collected from mares and foals and the colostrum collected from mares was used to measure anti-*Lawsonia intracellularis* specific IgG by IPMA as previously reported by Guedes and others (2002b). The rectal swabs collected from the mares were processed for nucleic acid purification within two hours of collection 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 (7900 HT; Applied Biosystem) for the presence of the aspartate ammonia lyase gene of *L intracellularis*, as previously reported by Pusterla and others (2008b).

No adverse reactions attributed to the vaccine or the administration procedure were detected among any vaccinated mares. All vaccinated and unvaccinated sentinel mares remained in good health, with normal appetite, attitude, rectal temperature and faecal character throughout the entire study period.

Molecular detection of *L intracellularis* was documented in three vaccinated mares. The faecal shedding was first detected 12 to 15 days following the intrarectal vaccine administration and lasted for one to three days after initial detection. All unvaccinated sentinel mares remained PCR negative for *L intracellularis* for the entire study period.

The vaccinated mares seroconverted 14 to 28 days following vaccination (mean [sd] 20·1 [5·8] days). The maximum titre reached by the eight mares ranged from 120 to 240. Antibodies specific for *L intracellularis* remained detectable (titre ≥60) for 42 to 70 days (59·5 [9·1] days) after vaccination (Fig 1). All sentinel unvaccinated mares remained seronegative for *L intracellularis* throughout the entire study.

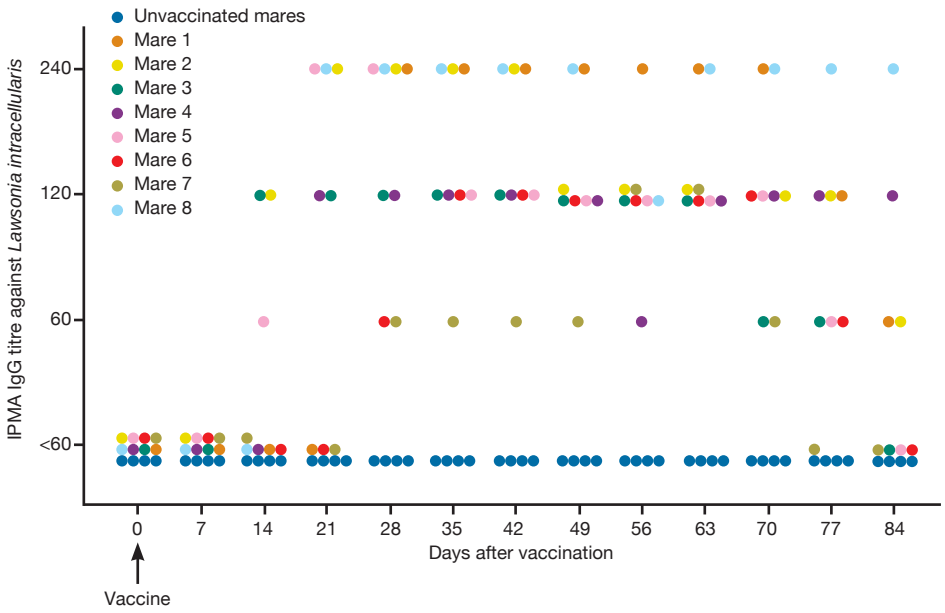

FIG 1: Serological response of 12 mares vaccinated with a modified-live avirulent *Lawsonia intracellularis* vaccine. Unvaccinated mares are the four unvaccinated sentinel mares. IPMA Immunoperoxidase monolayer assay

All mares foaled without any problems. The time from vaccination to foaling in the eight vaccinated mares ranged from 13 to 32 days (23·1 [6·8] days) (Table 1). The unvaccinated sentinel mares foaled 11 to 32 days (20·75 [8·7] days) following the beginning of the study. Colostral antibodies were detected in two of the eight seropositive mares with titres of 240 (Table 1). These two mares foaled 17 and 24 days following vaccination, at which times, measurable serum titres were 120 and 240, respectively. Two mares with no detectable antibodies in the colostrum foaled 13 and 21 days following vaccination, respectively. These two mares had first-time detectable serum antibodies eight days post-foaling and at the day of foaling, respectively. The remaining four mares with no detectable antibodies in the colostrum foaled 19, 28, 31 and 32 days after vaccination; these mares had first-time detectable serum antibodies five, seven, 17 and four days before foaling, respectively.

All foals had negative antibody titres (<60) against *L intracellularis* prior to colostrum ingestion. All foals had adequate passive transfer of colostral antibodies based on adequate IgG levels (≥800 mg/dl) using a commercial IgG test (SNAP Foal IgG Test; IDEXX Laboratories). Passive transfer of colostral antibodies against *L intracellularis* was documented in six foals with maximal titres ranging from 60 (in two foals), to 120 (in four foals) (Table 2). Passively acquired antibodies against *L intracellularis* remained detectable for 11 to 56 days (44·5 [19] days). The first foal with no detectable antibodies against *L intracellularis* post-colostrum ingestion was born from a mare with no detectable antibodies in serum and colostrum at the time of foaling. The second foal with no detectable colostral antibodies against *L intracellularis* was born from a mare with high serum and colostrum titre (240). Interestingly, a foal born from a seronegative mare that had measurable antibodies against *L intracellularis* for the first time on the foaling day had measurable antibodies against *L intracellularis* for 56 days following colostrum ingestion. All the foals born from unvaccinated sentinel mares remained seronegative throughout the eight-week study period.

Patterns of faecal shedding of the healthy mares were similar to a recent study performed in weanling foals, but at a lower magnitude (Pusterla and others 2008b). Faecal shedding was only observed in three of eight mares in the present study and lasted for a short period of time. The reason for the lower detection rate and shorter detection time is unclear, but may be related to the detection limit of the assay, as well as the larger volume of faeces produced by adult animals, meaning less organisms per gram of faeces. Similar to that seen in foals, the detection of *L intracellularis* 12 to 15 days following the intrarectal vaccine administration is highly suggestive of active mucosal replication of the organism. Immunohistochemical detection of *L intracellularis* in rectal mucosa biopsies was not attempted in the present study. All sentinel mares remained PCR negative for *L intracellularis* for the entire study period, indicating that they were not exposed to vaccinated herdmates or potential environmental sources. Although natural exposure to *L intracellularis* has been reported in adult horses, disease or faecal shedding has not yet been documented (Pusterla and others 2008a).

The results of the study are in agreement with recent work determining the humoral immune response in pigs and weanling foals after challenge with the attenuated vaccine strain of *L intracellularis* (Guedes and Gebhart 2003, Pusterla and others 2008b). The study reported here also supports previous work on adult sows naturally exposed to *L intracellularis* (Guedes and others 2002a), which found gilts developed high serum antibody titres that lasted up to three months in some animals. The absence of detectable seroconversion observed in the unvaccinated sentinel horses in the current study supports the lack of exposure from vaccinated herdmates or from potential environmental sources.

Colostral antibodies were detected in two of six seropositive mares at the time of foaling. To the knowledge of the authors, no prior study has documented the presence of colostral antibodies against *L intracellularis* in equine colostrum. Based on the results, it does not appear that serum IgG levels or the number of days of seropositivity before foaling correlate with colostrum antibodies. However, the results suggest that broodmares with undetectable serum antibodies will also have colostral antibodies below the detection limit.

The results reported here indicate that passive transfer of specific antibodies to *L intracellularis* commonly occurs following vaccination of mares. Of interest are the two foals with non-detectable antibodies after colostrum ingestion. While one of the two foals was born from a mare that foaled 13 days following vaccine administration and may not have had enough time to mount a peripheral immune response, the second foal was born from a mare with high peripheral

TABLE 1: Days from vaccination to foaling, days to positive titres from vaccination, days between positive titres and foaling, serum and colostrum titres at foaling and duration of detectable antibodies in eight mares vaccinated with a modified-live avirulent *Lawsonia intracellularis* vaccine

Mare	Days from vaccination to foaling	Days to positive titre from vaccination	Days between positive titre and foaling	Serum titre at foaling*	Colostrum titre	Duration of positive titre (days)
1	13	21	–8	<60	<60	63
2	19	14	+5	120	<60	70
3	17	14	+3	120	240	63
4	28	21	+7	120	<60	63
5	31	14	+17	240	<60	63
6	21	21	0	<60	<60	49
7	32	28	+4	60	<60	42
8	24	21	+3	240	240	63

* Immunoperoxidase monolayer assay positive titre ≥60
– Seroconversion occurred post-foaling, + Seroconversion occurred pre-foaling

TABLE 2: Titre pre- and post-colostrum ingestion and duration of detectable antibodies in eight foals born from mares vaccinated with modified-live avirulent *Lawsonia intracellularis* vaccine

Foal*	Titre pre-colostrum ingestion	Titre post-colostrum ingestion	Duration of positive titre (days)
1	<60	<60	0
2	<60	60	32
3	<60	60	11
4	<60	120	56
5	<60	120	56
6	<60	120	56
7	<60	120	56
8	<60	<60	0

* Foal number corresponds to mare in table 1

and colostrum antibody titres. The results from the second foal may be related to the previously mentioned variability in ingestion and/or absorption of specific colostral antibodies. It should also be mentioned that there was no clinicopathological evidence that passive transfer of colostral antibodies had failed in any of the study foals. Passive transfer of maternally derived antibodies against *L intracellularis* has been previously reported in pigs (Holyoake and others 1994, Guedes and others 2002a). These studies showed that piglets born from seropositive sows had low antibody titres against *L intracellularis* that usually lasted for only three weeks. Similar results were found in the study foals with measurable colostral antibodies against *L intracellularis* that remained detectable for 11 to 56 days.

Mucosal IgA, as well as the cell-mediated immune response, is more likely to be associated with protection against *L intracellularis* infection. More studies are needed to determine the presence of antibodies specific for *L intracellularis* in the colostrum of seropositive mares and to correlate those findings with immunity and protection in foals.

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