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Evaluation of the humoral immune response and fecal shedding in weanling foals following oral and intra-rectal administration of an avirulent live vaccine of *Lawsonia intracellularis*

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ABSTRACT

Equine proliferative enteropathy (EPE) caused by *Lawsonia intracellularis* has recently been recognized as an emerging disease in foals. Whilst the clinical entity, diagnostic evaluation and treatment of affected foals have been well established and described, preventive measures for EPE have remained largely unaddressed. The objectives of this study were to investigate the humoral immune response and onset and duration of fecal shedding in foals after oral and intra-rectal administration of a modified-live vaccine of *L. intracellularis*. Foals were vaccinated twice, 3 weeks apart, via oral drenching after pre-medication with a proton-pump inhibitor (omeprazole; group 1), intra-rectally (group 2) or orally without any pre-medication (group 3). The health status of the foals was monitored daily, with feces and serum collected at regular intervals for Polymerase Chain Reaction (PCR) and serology.

All foals remained healthy and no adverse vaccine reactions were observed. Fecal shedding lasted from 1 to 12 days and was mainly detected in foals receiving the intra-rectal vaccine 11–15 days following the first vaccine administration. Serological responses were measured in the majority of the vaccinated foals. All foals vaccinated intra-rectally seroconverted after the first vaccine, compared to 50% and 0% of foals in groups 1 and 3, respectively. Pre-medication with omeprazole prior to oral vaccination in group 1 foals led to an earlier and stronger detectable humoral response compared to non pre-medicated foals.

Introduction

Lawsonia intracellularis, an obligate intracellular organism, is the etiological agent of proliferative enteropathy in a number of domestic and wild animal species (Lawson and Gebhart, 2000). The enteric disease is characterized by the proliferation of immature epithelial cells of the crypt of the distal small and/or proximal large intestine (Smith and Lawson, 2001). This pathological process leads to macroscopic thickening of the mucosa that results in the enteric signs often associated with the disease.

Equine proliferative enteropathy (EPE) is considered an emerging disease and has been reported with increasing frequency in North America, Europe, Australia and more recently in South Africa (Anonymous, 2007; Lavoie and Drolet, 2007). EPE commonly affects weanling foals and has a sporadic occurrence, although outbreaks on breeding farms have been reported (Lavoie et al., 2000). Affected weanlings commonly show rapid weight loss, lethargy, depression, fever, subcutaneous edema, diarrhea and colic. An ante mortem 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. 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 feces from free-living or domestic animals (Lawson and Gebhart, 2000; Lavoie and Drolet, 2007).

Treatment of EPE involves the administration of effective antimicrobial agents and supportive care. Although the clinical entity, diagnostic work-up and treatment of EPE are well established and described, preventive measures for the disease have remained largely unaddressed. Prevention has been best described in pigs using in-feed antimicrobials and a commercially available *L. intracellularis* modified-live vaccine (Lawson and Gebhart, 2000; Guedes and Gebhart, 2003; Kroll et al., 2004; Almond and Bilkei, 2006; McOrist

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and Smits, 2007). No information regarding the use of the *L. intracellularis* vaccine in horses is available. The objective of this study was therefore to investigate the humoral immune response and onset and duration of fecal shedding in healthy foals following selected methods of administration of an avirulent modified-live vaccine of *L. intracellularis*.

Materials and methods

Animals

Fifteen clinically healthy Quarter Horse weanling foals, comprising seven fillies and eight colts between the ages of 4 and 6 months, were used for the study. The foals belonged to the research herd at the Center for Equine Health, University of California at Davis. The herd has had no history or recorded cases of EPE. All the foals were evaluated for any signs of ill health by means of a full physical examination and a complete blood cell count performed at the beginning of the study and all results were within reference intervals.

Four days before immunisation, serum samples were collected from all foals and tested for *L. intracellularis* by immunoperoxidase monolayer assay (IPMA; Guedes et al., 2002) in order to document a seronegative status in each foal. In addition, fecal samples were collected and tested for *L. intracellularis* DNA by real-time polymerase chain reaction (PCR) in order to document the absence of fecal shedding. The foals were randomly assigned to three groups of five foals each. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California. Due to the small number of foals per group, only descriptive data are presented.

Study design, immunization protocol and sample collection

Each group was kept in a separate dirt paddock for the entire study period of 42 days. The foals had free choice of grass and alfalfa hay and water and were supplemented daily with a commercial foal supplement.

For each group, 4/5 foals received 50 mL of the modified-live avirulent *L. intracellularis* vaccine (Enterisol Ileitis, Boehringer Ingelheim Vetmedica) given twice, 3 weeks apart, while one foal in each group served as an unvaccinated sentinel. All vaccines originated from the same batch (batch number 153 C). The frozen vaccine was handled and thawed in accordance with label instructions. Foals of group 1 were pre-medicated with 4 mg/kg of omeprazole (GastroGard, Merial) given orally once a day for 3 days prior to each oral administration of the vaccine in an attempt to increase gastric pH and prevent degradation of the avirulent *L. intracellularis* in the stomach. Group 2 foals were vaccinated intra-rectally using a 12 Fr, 20 cm urinary catheter (Tyco Healthcare). Finally, foals of group 3 received the oral vaccine without any pre-medication. All oral vaccines were applied directly to the caudal portion of the oral cavity of the foals by use of a sterile plastic 60 mL syringe.

All foals were observed daily for general attitude and appetite. Further, a complete physical examination was performed every other day for the entire study period. Serum was drawn from each foal once weekly, while rectal swabs using rayon tipped applicators (Puritain Medical Products Company LLC) were collected every other day. Additional nasal and fecal swabs were collected from all foals at the beginning of the study and 3 weeks following each vaccine administration in an attempt to document the presence of mucosal immunoglobulin (Ig) A antibodies against *L. intracellularis*.

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 and IgA antibodies by IPMA, as previously reported (Guedes, 2002; Guedes et al., 2002). The rectal swabs were processed for nucleic acid purification within 2 h of collection. One milliliter of phosphate buffered saline (PBS) was added to each swab in a conical tube. Thereafter, each sample was vortexed for 10 s and centrifuged at 13,000 *g* for 60 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) according to the manufacturer's recommendations. The purified DNA was then analysed by real-time PCR for the presence of the aspartate ammonia lyase gene of *L. intracellularis*, as previously reported (Feary et al., 2007). Positive (DNA from cell grown *L. intracellularis*) and negative (*L. intracellularis*-free DNA from fecal samples) DNA controls were used with each run. Mucosal IgA immune response was measured from nasal and rectal swabs (Guedes, 2002). Briefly, 96-well plates containing acetone fixed monolayer of McCoy cells highly infected with *L. intracellularis* were rehydrated in a solution of PBS with 5% skimmed milk for 10 min at 37 °C to block nonspecific reactions. The swabs were diluted in the same block solution in serial four-fold dilutions. Then, 50 μL of each diluted sample was added to the test well. The plate was incubated for 30 min at 37 °C, then washed five times with phosphate buffered saline with 0.05% Tween 20 (PBST). Goat anti-equine IgA-peroxidase conjugate (Bethyl Laboratories), diluted 1:1000 in PBST, was added at a concentration of 30 μL/well, and incubation proceeded for 45 min at 37 °C. The plate was washed five times with PBST and 100 μL pre-diluted chromogen (3-amino-9-ethyl-carbazole; AEC; A-6926; Sigma) solution were added to each well and incubated at room temperature for 20 min. The plate was washed with PBS three times, allowed to dry, and examined using an inverted light microscope.

Results

There were no detectable adverse reactions attributed to the vaccine or administration procedures among any vaccinated foals. All vaccinated and sentinel foals remained in good health, with normal appetite, attitude, rectal temperature and fecal character throughout the entire study period. The concentration of total solids in the serum of all foals remained within normal limits (range 5.7–6.5 g/dL) throughout the study period.

Molecular detection of *L. intracellularis* was not documented in any of the vaccinated foals of group 1 throughout the entire study period (Fig. 1A). This was in sharp contrast to the PCR positive rectal swabs found in all vaccinated foals of group 2. The fecal shedding was first detected 11–15 days following the first intra-rectal vaccine administration and lasted for 1–12 days after initial detection (Fig. 1B). Fecal shedding of *L. intracellularis* in the vaccinated foals of group 3 was only documented in the first 2 days following the first oral vaccine administration in 3/4 vaccinated foals (Fig. 1C). All sentinel foals remained PCR negative for *L. intracellularis* for the entire study period.

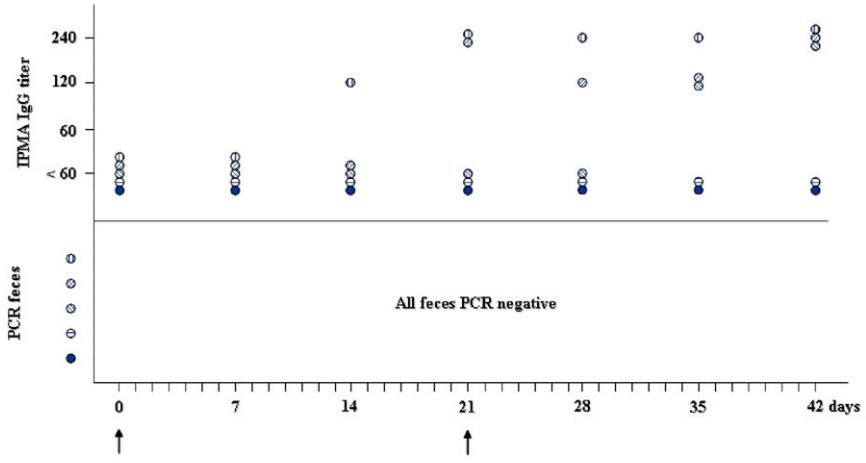

Fig. 1A. Serological response and onset and duration of fecal shedding of foals from group 1 (oral vaccine with omeprazole pre-medication). Open circles represent vaccinated foals, solid circles represent unvaccinated sentinel horse. Arrows indicate vaccination points. IgG titers ⩾ 60 against *L. intracellularis* are considered positive by IPMA.

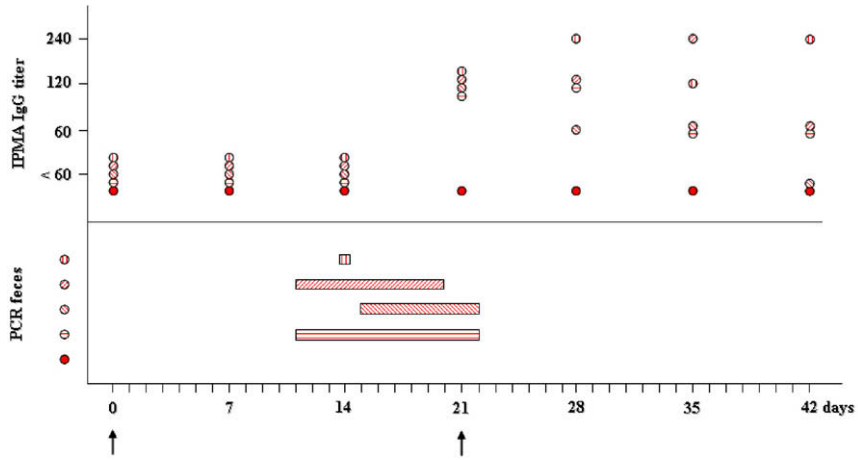

Fig. 1B. Serological response and onset and duration of fecal shedding of foals from group 2 (rectal vaccine). Open circles represent vaccinated foals, solid circles represent unvaccinated sentinel horse. Arrows indicate vaccination points.

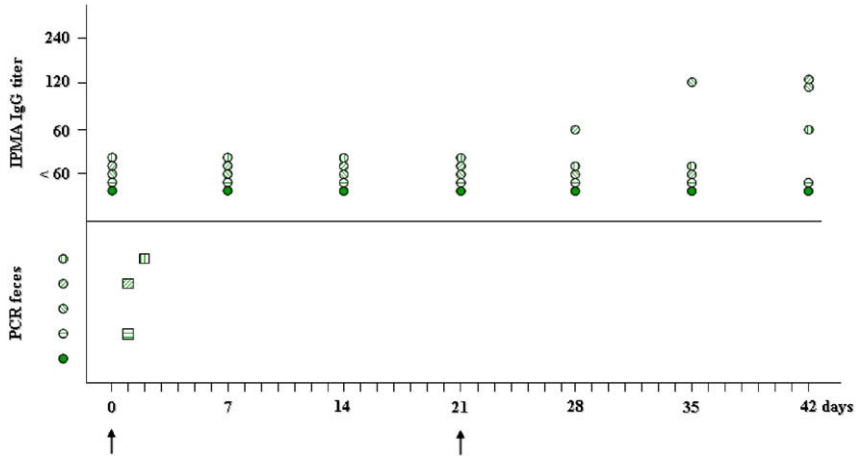

Fig. 1C. Serological response and onset and duration of fecal shedding of foals from group 3 (oral vaccine without omeprazole pre-medication). Open circles represent vaccinated foals, solid circles represent unvaccinated sentinel horse. Arrows indicate vaccination points.

Two foals from group 1 had measurable IgG titers against *L. intracellularis* at 21 days and three foals at 42 days (Fig. 1A). One foal did not seroconvert throughout the entire study time. The titers measured in the foals of group 1 ranged from 120 to 240. All four vaccinated foals from group 2 seroconverted by day 21 (Fig. 1B) with titers of 120. Up to day 42, the titer of these foals ranged from 60 to 240 and one foal reverted back to a titer below the cut-off by day 42. Foals of group 3 had a delayed onset of seroconversion (Fig. 1C) and only three foals were seropositive between 28 and 42 days. IgG serum antibody titers of foals of group 3 ranged from 60 to 120 and one foal never seroconverted throughout the study period. All sentinel foals remained seronegative for *L. intracellularis* for the entire study.

Serum IgA against *L. intracellularis* was only detected from two foals of group 2 on days 21 and 28, respectively. One of the two foals also had detectable nasal IgA on day 42. None of the rectal swabs had detectable IgA against *L. intracellularis* during the entire study period.

Discussion

Recent studies have shown that the oral administration of the attenuated *L. intracellularis* vaccine in pigs resulted in substantial protection against proliferative enteropathy (Kroll et al., 2004; McOrist and Smits, 2007). The vaccine volume used in this study was chosen based on unpublished safety data (R.M. Stenbom, personal communication) and in an attempt to adjust the porcine dose (2 mL) to the average weight of a 5 month old Quarter Horse foal. Mucosal immunisation regimes that employ the oral routes of delivery are often compromised by antigen degradation in the stomach. We hypothesised that the low stomach pH of horses may inactivate the attenuated vaccine strain. In an attempt to potentially increase the viability and integrity of the orally administered organisms, the foals of group 1 received a proton-pump inhibitor prior to vaccine administration in order to increase their stomach pH and allow an unaltered passage of *L. intracellularis* through the stomach. Immunization by alternative routes including the intra-rectal and intra-nasal route is becoming increasingly recognized in large animals for generating protective antibody responses at mucosal surfaces (Sedgmen et al., 2004). In an attempt to bypass the stomach, but still allow for mucosal contact of the vaccine strain, we investigated the intra-rectal administration of the vaccine in foals of group 2.

The absence of clinical signs and hypoproteinemia in the study foals following the administration of the modified-live *L. intracellularis* vaccine is in agreement with previously reported studies in pigs (Guedes and Gebhart, 2003; Kroll et al., 2004). These findings support the safety of the vaccine and the inability of the attenuated *L. intracellularis* vaccine strain to potentially induce proliferative lesions severe enough to cause disease.

Molecular detection of *L. intracellularis* was mainly documented in foals following intra-rectal administration of the vaccine strain. In these foals, the shedding started in the second week following the first vaccine administration and is, therefore, likely to represent true pathogen replication occurring in the lower gastrointestinal tract. Immunohistochemical detection of *L. intracellularis* in the rectal mucosa will be necessary in the future to better document and define the host-pathogen interactions at the site of vaccine administration. Fecal shedding of *L. intracellularis* detected in the vaccinated foals of group 3 in the first 2 days following the first oral vaccine administration most likely represents challenged *L. intracellularis* organisms that were expelled via feces following normal gastrointestinal transit.

Our results are in agreement with a recent study evaluating the protective immunity in pigs following oral administration of the avirulent live vaccine (Kroll et al., 2004). In that study, vaccinates did not shed *L. intracellularis* during a 3 week period after vaccination. The lack of detectable *L. intracellularis* in foals administered the oral vaccine is probably caused by the reduced capacity of the avirulent bacterial isolate to replicate in the host. On the other hand, our results are in contrast to a recent study evaluating the onset and duration of fecal shedding in pigs after challenge with the attenuated vaccine strain of *L. intracellularis* (Guedes and Gebhart, 2003). In that study, fecal shedding was initially detected 2 weeks following and lasted, intermittently, 9 weeks after exposure to the vaccine. The discrepancy between the two pig studies is unclear, but may have been associated with the age of the vaccinated pigs, type of housing, the vaccine administration protocols, or variations in virulence of the live vaccine strain. Based on our results, it appears that the live avirulent *L. intracellularis* strain administered orally in foals of group 1 and 3 is not likely to have great potential to contaminate the environment and eventually spread to other foals via feces.

All sentinel foals remained PCR negative for *L. intracellularis* for the entire study period, indicating no exposure from vaccinated herdmates or potential environmental sources. However, it is possible that the long-term presence of *L. intracellularis* in the feces of foals vaccinated via the intra-rectal route may become a source of infection for naïve herd mates, especially when overcrowding and fecal contamination of feed and water is present. Traditionally, genomic and proteomic analysis of *L. intracellularis* of porcine origin have shown great homogeneity between strains, making a differentiation between field and vaccine isolates very difficult (McOrist et al., 1995). However, recent work using multiple-locus variable number tandem repeat analysis has shown promise in differentiating *L. intracellularis* isolates of porcine and equine origin (C. Gebhart, personal communication). Such an approach could be used to differentiate vaccine versus field strains in previously vaccinated foals that develop enteric signs compatible with EPE.

Serological responses were measured in the majority of the foals from each of the three groups. Vaccinated foals from groups 1 and 2 showed comparable titers at 42 days however, all foals receiving the intra-rectal vaccine seroconverted after the first vaccine, compared to only 50% and 0% of foals of group 1 and 3, respectively. It would have been interesting to study the serological responses of one versus two vaccine administrations in an attempt to determine if multiple vaccine administrations are necessary to document seroconversion in the majority of the foals.

It also appeared that pre-medication with omeprazole prior to oral vaccination led to an earlier and stronger measurable humoral response in foals of group 1 when compared to the foals that did not receive any gastroprotectant. This implies that the increase in stomach pH may be beneficial for either the viability or the integrity of the avirulent *L. intracellularis* vaccine strain. Necropsy with evidence of *L. intracellularis* in crypt epithelial cells was not attempted in this study and would have been necessary to document active multiplication of the organisms in the small intestine of vaccinated foals of group 1.

Our study is in agreement with recent work determining the humoral immune response in pigs after challenge with the attenuated vaccine strain of *L. intracellularis* (Guedes and Gebhart, 2003). In that study, humoral response was initially detected 5 weeks after vaccine exposure with titers ranging from 30 to 480. The earlier humoral response documented in the vaccinated foals when compared to pig data could be explained by the difference in vaccine dose (i.e. 25 × higher dose in foals versus pigs) and the route of administration (drinking water for pigs versus oral drenching and intra-rectal application in foals).

Immunological responses against *L. intracellularis* during infection have remained mostly uncharacterised (Smith and Lawson, 2001). Secretory IgA and cell-mediated immune response are likely to play a role at overcoming natural infection with *L. intracellularis*. Although cell-mediated immune response was not investigated in this study, the use of anti-*Lawsonia intracellularis* IgG levels in peripheral blood following oral and intra-rectal vaccine administration may complement and indirectly assess antigen-specific mucosal immunity. The level of humoral response observed in all vaccinated foals was similar to the response observed in naturally infected animals (Lavoie et al., 2000; Pusterla et al., 2008). Although secretory antibodies may play a role in conferring protection towards natural *L. intracellularis* infection, the authors were unable to detect such immune response in the majority of the vaccinated foals. The lack of measurable mucosal immune response may have been linked to collection issues (small amount of mucosal secretions per sample) or the presence of inhibitory substances (proteases) within nasal and fecal secretions.

Conclusions

While this work represents an initial attempt at characterising the humoral immune response and onset and duration of shedding in foals exposed to an attenuated vaccine strain of *L. intracellularis*, the efficacy of this approach and potential for improvement of the wellbeing of weanling foals needs to be further investigated via experimental challenge studies or field efficacy trials. Promising data from field trials in pigs have shown that the use of *L. intracellularis* vaccine does not only prevent porcine proliferative enteropathy, but might result in more resistance to and tolerance against other infectious and management caused losses (Almond and Bilkei, 2006; McOrist and Smits, 2007).

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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