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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.

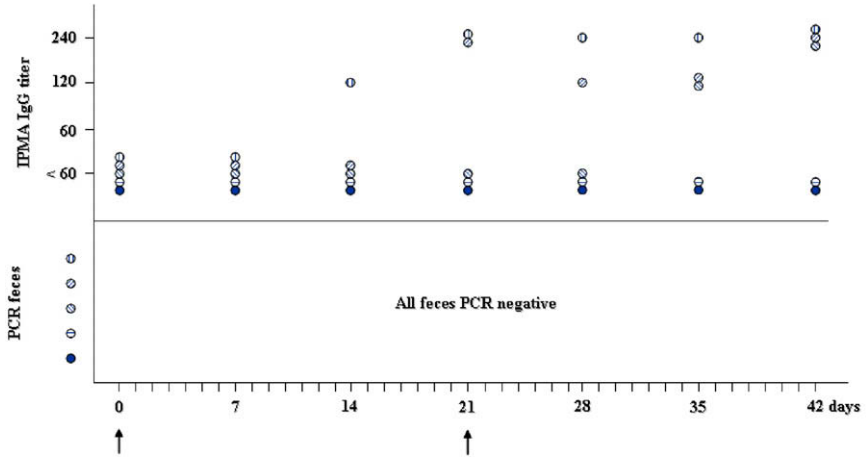

Fig. 1A. Serological response and onset and duration of fecal shedding of foals from group 1 (oral vaccine with omeprazole pre-medication). Open circles represent vaccinated foals, solid circles represent unvaccinated sentinel horse. Arrows indicate vaccination points. IgG titers ⩾ 60 against *L. intracellularis*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