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Original Research

The Possible Role Mares Play in the Epidemiology of Equine Proliferative Enteropathy

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ABSTRACT

The epidemiology of equine proliferative enteropathy (EPE), caused by the bacterium *Lawsonia intracellularis*, is poorly characterized. It has been suggested that horse-to-horse transmission of the bacterium may be possible, although no work has examined the role mares may play in the epidemiology of EPE. The goal of this study was to determine whether seropositive mares were more likely to have seropositive foals and whether seropositive mares were more likely to have foals with clinical or subclinical EPE. Serum samples were analyzed using an equine enzyme-linked immunosorbent assay for *L. intracellularis*–specific antibodies. Over 2 years (2012–2013 and 2013–2014), a total of 197 mare and foal pairs from two central Kentucky Thoroughbred farms with endemic EPE participated in this study. All foals were weaned by the end of October of their respective foaling year. There was no overall effect of mare serologic status on the occurrence of presumptive clinical or subclinical EPE in their offspring. Chi-square analysis determined that those mares with five or more seropositive months through October had a significantly higher number of foals with evidence of exposure to the bacterium between July and January ($P = .022$). Further, mares with less than five seropositive months through October of their foaling year were found to have foals 2.037 times less likely to be seropositive from July through January than those foals from those with five or more seropositive months through October. Additionally, there appeared to be a protective effect of mares on nursing foals with respect to exposure during the summer months.

1. Introduction

Lawsonia intracellularis is the causative agent of equine proliferative enteropathy (EPE) [1], a disease typically seen in weanlings and young yearling horses during the fall and early winter months [2]. The most common clinical signs observed with EPE include anorexia, fever, depression, dependent edema, colic, and diarrhea. Antemortem diagnosis is based on the presence of clinical signs, hypoproteinemia and hypoalbuminemia, and thickened small intestinal segments detected via abdominal ultrasound. Additional antemortem tests for the detection of *L. intracellularis* infection include detection of the pathogen shed in feces via an *L. intracellularis*–specific polymerase chain reaction (PCR) assay and analysis of serum samples for bacterium-specific antibodies.

With respect to the epidemiology of *L. intracellularis*, a fecal-oral route of infection is suspected [3], and what little

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else is known has been extrapolated from porcine proliferative enteropathy (PPE). In addition to horses, pigs, and hamsters, studies have revealed the presence of *L. intracellularis* in numerous species [4–7]. Thus far, attempts to incriminate any of these species in the spread or maintenance of *L. intracellularis* within susceptible populations have only been suggestive and not proven conclusively; this includes a recent study in which weanling horses were challenged with feces from *L. intracellularis*–infected rabbits [8]. In that study, the challenged horses failed to develop EPE; however, they did seroconvert after challenge. Work with PPE has implicated subclinically affected pigs in the spread of the bacterium in swine operations [9], and work with EPE has suggested a role for clinically affected horses [3]. Further, some have suggested the potential for sows to harbor and transmit the bacterium to their offspring [10], an aspect which has never been examined for EPE. Given that most Thoroughbred foals are cohoused in stalls with their respective mares for multiple hours per day for 4–6 months after foaling, as well as the propensity for foals to exhibit coprophagic tendencies, it seems tenable that mares could act as asymptomatic shedders of the bacteria and expose their foals to *L. intracellularis* before weaning.

The goal of this study was to use monthly serum sampling of mares and foals on two EPE endemic farms to detect seroconversion to *L. intracellularis* and determine whether mares show signs of exposure to the bacterium before their foals. Additionally, we sought to determine whether seropositive mares were more or less likely to have seropositive or EPE-affected offspring. Should we show mares play a role in the exposure of foals to *L. intracellularis*, this finding would provide new insight into the epidemiology of EPE and warrant further examination of potential steps for mitigation of this exposure as a means of decreasing EPE.

2. Materials and Methods

2.1. Study Farms and Horses

This study took place over two consecutive years from January 2012 to January 2013 (year 1) and January 2013 to January 2014 (year 2). During year 1, a Thoroughbred farm in central Kentucky, located within 20 miles of the University of Kentucky, was enrolled (farm A). For year 2, farm A was once again enrolled, while a second farm (farm B), meeting the same inclusion criteria, was also included. All mare and foal pairs that foaled on the two farms were used for this study. Both farms were considered endemic for EPE as they experienced one or more cases of clinical EPE in each of the last three or more years. Farms without a history of EPE were not included in this study as the goal of this project required cases of clinical and subclinical EPE. An approval to conduct this study was obtained from the University of Kentucky's Institutional Animal Care and Use Committee, and informed consent for inclusion of privately owned horses was obtained from either the farm veterinarian or the farm manager, both of whom were authorized to act on behalf of private owners.

2.2. Sample Collection, Processing, and Storage

The procedures used for this project are similar to those that have been reported previously [11]. Briefly, within 48 hours of parturition, whole blood samples were collected from the mare and newborn foal. After collection, samples were stored at 4°C on the farm until they were returned to University of Kentucky for centrifugation (800g × 10 minutes) followed by freezing of serum at −20°C. After the initial collection, samples were collected approximately every 4 weeks from each mare and foal through the middle of July, as described previously. Starting at the end of July, all study horses (mares and foals) had blood samples collected during the same 3-day period every 4 weeks to ensure that all horses were sampled during the same time period. Samples were handled as mentioned previously except that foal serum samples were held at 4°C for no more than 4 days before all samples were analyzed each month, as mentioned in the following, whereas mare samples were frozen at −20°C. After the final collection of blood samples in January, samples from each mare were thawed and run on the same plate using an *L. intracellularis*–specific enzyme-linked immunosorbent assay (ELISA).

2.3. Detection of L. intracellularis–Specific Antibodies

Serologic status in regards to *L. intracellularis* was determined using the ELISA method, as previously described [11,12]. A logarithmic regression line from a standard curve of a known immunoperoxidase monolayer assay (IPMA) titer sample was generated on each plate to allow for the conversion of tested sera to ELISA units (EU). A positive cutoff of 55 EU or greater was used based on previous work [12].

2.4. Classification of Foal EPE Status

At the end of the study, foals were placed into four categories based on their *L. intracellularis* and/or EPE status from October to February, as previously described [11]: presumptive clinical EPE, presumptive subclinical EPE, seropositive only, or seronegative. Given that definitive diagnosis of EPE can only be made at necropsy, all diagnoses of EPE were considered presumptive. All study horses were closely monitored on their farms daily for signs of illness, including clinical signs compatible with EPE. Any horses with suspected EPE were reported to the investigators and samples for EPE diagnosis submitted. Monthly fecal PCR testing was not undertaken during this study as a means of bacterial detection because the results can be highly variable [13–16], and previous epidemiologic work with EPE was not augmented by its addition [17].

2.5. Data Analysis

Chi-square (SigmaStat, SPSS Inc, Chicago, IL) analysis was used to determine whether mare serologic status had an effect on the occurrence of EPE. Chi-square and odds ratio analyses were used to determine whether the number of seropositive months a mare experienced had a significant effect on seroconversion in her foal. Five months