



RESEARCH ARTICLE

Seroprevalence against *Lawsonia intracellularis* among Thoroughbred Horses in Korea

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Received: Apr 24, 2024

Accepted: Aug 5, 2024

KeywordsLawsonia intracellularis
Seroprevalence
South Korea
Thoroughbred horse***Corresponding Author:**

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ABSTRACT

Lawsonia intracellularis is the etiological agent behind equine proliferative enteropathy (EPE), recently identified and emerging intestinal ailment in horses. *Lawsonia intracellularis* is an obligate intracellular, curved, gram-negative bacterium that resides within the apical cytoplasm of infected intestinal enterocytes. Although serological studies on EPE have been conducted in several countries, the prevalence of *Lawsonia intracellularis* in Korea has not been reported. This study aimed to examine the seroprevalence of *Lawsonia intracellularis* among healthy Thoroughbred horses in Korea. In this study, 180 healthy adult Thoroughbred horses (≥ 2 years of age) and 8 foals (< 2 years of age) were sampled from each of the four Korean provinces. Antibodies against *Lawsonia intracellularis* were assessed using a blocking enzyme-linked immunosorbent assay. Among adult horses, 78.9% (142/180) tested positive, 20% (36/180) yielded inconclusive results, and 1.1% (2/180) tested negative. Among foals, 87.5% (7/8) tested positive with 12.5% (1/8) yielding inconclusive data. The findings of this study hold significant implications in identifying the serological infection status of *Lawsonia intracellularis* among domestically bred Thoroughbred horses and establishing fundamental data regarding this infection in Korea.

INTRODUCTION

Lawsonia intracellularis is the etiological agent of equine proliferative enteropathy (EPE) or equine ileitis, a recently identified and emerging intestinal disease in horses, primarily affecting weaning foals (Frazer, 2008; Lawson and Gebhart, 2000). *Lawsonia intracellularis*, an obligate, curved, motile, gram-negative rod bacterium, predominantly resides within the apical cytoplasm of infected intestinal enterocytes, specifically in the ileum and distal jejunum (Frazer, 2008; Lawson and Gebhart, 2000; Arroyo et al., 2013; Lavoie et al., 2000). The pathogen triggers the proliferation of infected enterocytes, leading to thickening of the small intestine and occasionally the large intestine (Atherton and McKenzie, 2006; Sampieri et al., 2006; Merlo et al., 2009).

Lawsonia intracellularis can only be cultured in vitro in a specific cell culture environment (Lawson and Gebhart, 2000). Apart from horses, *Lawsonia intracellularis* infects many species of domestic and wild animals, including pigs, hamsters, rabbits, foxes, deer, ferrets, ostriches, and nonhuman primates (Pusterla and Gebhart, 2013). EPE was initially reported in horses in 1982 by Duhamel and Wheeldon (Duhamel and Wheeldon, 2013). Since 1996, numerous instances of sporadic cases and outbreaks in breeding farms have been reported (Williams et al., 1996; Frank et al., 1998; Brees et al.,

1998; Schumacher et al., 2000; Lavoie et al., 2000; Bihu, 2003). In recent years, there has been a notable escalation in the reported cases of EPE predominantly affecting postweaning foals and sporadically adult horses (Pusterla and Gebhart, 2013). The incidence of this disease has nearly attained global proportions and has been documented in the USA (Merlo et al., 2009), Canada (Lavoie et al., 2000), Europe (Allen et al., 2009; Deprez et al., 2005), Israel (Steinman et al., 2014), Brazil (Gabardo et al., 2015), and Australia (McClintock and Collins, 2004).

Anorexia, pyrexia, peripheral edema, rapid weight loss, lethargy, rough hair coat, colic, and diarrhea are among the most common clinical findings observed in affected foals (Frazer, 2008; Lavoie et al., 2000; Atherton and McKenzie, 2006; Sampieri et al., 2006). Although diarrhea is frequently observed in affected foals and can vary from cow pie to watery consistency, some affected foals may exhibit normal fecal characteristics. Foals with EPE may also present with concurrent disorders such as respiratory tract infections, gastric ulcerations, intestinal parasitism, bacterial infections (*Clostridium* spp., *Salmonella* spp., *Rhodococcus equi*, *Neorickettsia risticii*), rotavirus and coronavirus infections, intestinal obstruction, intoxication with plants and chemicals, and exposure to pharmacologic agents such as nonsteroidal anti-inflammatory drugs or antimicrobials (Pusterla and Gebhart, 2013).

The most consistent laboratory finding in clinical EPE is leukocytosis and hypoproteinemia, primarily induced by severe hypoalbuminemia (Frazer, 2008; Merlo et al., 2009). Typically, total protein levels are approximately 5.0 g/dL, with albumin concentrations usually measuring at 2.0 g/dL. In a recent case report involving 57 affected foals, hypoalbuminemia emerged as the sole consistent clinicopathologic abnormality among them, with albumin concentrations ranging from 0.9 to 3.3 g/dL (normal reference range 2.7 to 4.2 g/dL) (Frazer, 2008). Additionally, affected foals might present with nonspecific blood abnormalities such as anemia or hemoconcentration, leukocytosis or neutropenia, hyperfibrinogenemia, elevated muscle enzyme activity, and electrolyte imbalances (Frazer, 2008).

The gold standard for diagnosis is postmortem histopathology; however, antemortem diagnosis is feasible through polymerase chain reaction (PCR) detection of *Lawsonia intracellularis* in fecal samples or rectal swabs and/or serology (Pusterla et al., 2009).

At present, in South Korea, there are approximately ~1,000 donkeys and ~27,000 domestic horses, comprising 12,000 Thoroughbreds, 1,000 individuals from other horse breeds (e.g., Warmbloods and Quarter horses), and 14,000 native horses (specifically the Jeju Halla horse), of which approximately ~5,000 are Jeju horses designated as natural monument No. 347 by the government (Shin et al., 2020). Among the diseases posing challenges to foals raised in Korea, prominent ones include diarrhea, pneumonia, and neonatal isohemolytic diseases. Foal diarrhea typically manifests acutely within 3 days of birth due to *Clostridium perfringens*. Foal pneumonia is mainly caused by *Rhodococcus equi* when immune antibodies decline. Newborn jaundice occurs within 3 days of birth due to differences in the blood type between parent (mare and stallion) horses.

Serological studies of EPE have been conducted in several countries (Lavoie et al., 2000; Merlo et al., 2009; Allen et al., 2009; Deprez et al., 2005; Steinman et al., 2014; Gabardo et al., 2015; McClintock and Collins, 2004); however, the prevalence of *Lawsonia intracellularis* in Korea has not been investigated. Therefore, to address this gap in the literature, this study aimed to assess the seroprevalence of *Lawsonia intracellularis* among healthy Thoroughbred horses in Korea.

MATERIALS AND METHODS

Sampling

In this study, a total of 180 healthy adult Thoroughbred horses (≥ 2 years of age) and 8 foals (< 2 years of age) residing in each of the four provinces of Korea were sampled. Blood samples were collected via jugular venipuncture into plain tubes, and the serum was subsequently separated by centrifugation, and then stored frozen at -20°C until analysis.

Blocking Enzyme-Linked Immunosorbent Assay

Antibodies against *Lawsonia intracellularis* were evaluated using a blocking enzyme-linked immunosorbent assay (bELISA, SVANOVR® *L. intracellularis*/Ileitis-Ab, Sweden) conducted with an enzyme-linked immunosorbent assay (ELISA) analyzer (Infinite M200PRO, TECAN, AUSTRIA). The results were interpreted in accordance with the methods outlined by Loublier *et al.* (2020). A specific formula was applied to convert the absorbance values obtained into a calculated percentage of inhibition (PI). For the purpose of this study, PI values below 20% were classified as negative (N), those ranging from 20 to 30% as inconclusive (I), and those exceeding 30% as positive (P), indicating the presence of antibodies against *Lawsonia intracellularis*.

RESULTS

Province-wise results of the bELISA analysis are presented in Table 1. The detection of antibodies against *Lawsonia intracellularis* via bELISA yielded positive results for 180 Thoroughbred horses (79.3%). Among the adult horses, 78.9% (142/180) tested positive, 20% (36/180) yielded inconclusive results, and 1.1% (2/180) tested negative. Among the foals, 87.5% (7/8) tested positive, whereas 12.5% (1/8) provided inconclusive data.

Table 1: Blocking ELISA serology results obtained from 180 healthy horses residing in the four Korean provinces

Province (age)	bELISA Serology Results (**No. of Horses)		
	Negative (<20% *PI)	Inconclusive (20–30% PI)	Positive (>30% PI)
Gumi (Adult/Foal)	0/0	14/0	12/0
Jang Soo (Adult/Foal)	2/0	12/0	33/0
Jeju (Adult/Foal)	0/0	0/1	98/7
Youngcheon (Adult/Foal)	0/0	10/0	10/0
Total (%)	2(1.1)/0(0.0)	36(20.0)/1(12.5)	142(78.9)/7(87.5)
*PI; percentage of inhibition			
** Number of horses.			

DISCUSSION

A presumptive diagnosis of EPE is established based on the age of the affected animal, clinical signs, hypoproteinemia, hypoalbuminemia, and the presence of thickened small intestinal loops observed during ultrasonographic evaluation, following the exclusion of other causes of enteropathy and protein loss (Loublier *et al.*, 2020). Abdominal ultrasonography, although not very sensitive, may show segments of thickened small intestine and an excess of abdominal fluid (Loublier *et al.*, 2020). A confirmed antemortem diagnosis achieved through the PCR detection of *Lawsonia intracellularis* in feces or rectal swabs, and/or serology (Pusterla *et al.*, 2009). Serology serves as a valuable tool for detecting antibodies against *Lawsonia intracellularis*. However, its drawback lies in the fact that detectable antibodies typically emerge two weeks after the onset of the disease (Gebhart, 2006; Jacobson *et al.*, 2004). Therefore, this method only offers insights into previous exposure and dose not necessarily correlate with clinical disease (Gebhart, 2006; Jacobson *et al.*, 2004; Guedes *et al.*, 2004). Various serologic assays, such as the indirect fluorescent antibody test, ELISA, and immunoperoxidase monolayer assay (IPMA), have been validated for pigs. However, a preliminary

comparative study utilizing equine serum samples demonstrated that IPMA is the most accurate among all serologic tests in determining the presence of specific anti-*Lawsonia intracellularis* antibodies in foals with EPE. However, the limited availability of horse samples presents challenges in conducting such tests (Wattanaphansak et al., 2008; Keller and Schroeder, 2005).

ELISA holds an advantage in its ease of application for large sample sizes (Boesen et al., 2005). An adaptation of a porcine ELISA test has been validated and used to determine seroprevalence in Thoroughbred farms in Kentucky (Wattanaphansak et al., 2008; Page et al., 2011). Hassenin et al. (2017) have affirmed that bELISA testing serves as a useful tool for antibody detection in horses. In this study, antibodies against *Lawsonia intracellularis* were detected through bELISA in 78.9% (142/180) of the adult horses and 87.5% (7/8) of foals. When compared with findings from other investigations (ranging from 9.4% to 100%), our results exhibit some variation (Kranenburg et al., 2011; Guimaraes-Ladeira et al., 2009; Steinman et al., 2014; Page et al., 2014; Pusterla et al., 2008). This discrepancy could stem from variations in applied methods and data analyses. However, our study yielded a positivity rate of 78.9%, which aligns closely with the 98.8% positivity reported by Loublier et al. (2020) in adult horses. The notable seroprevalence observed among adult horses in our study suggests either the prolonged persistence of antibodies against *Lawsonia intracellularis* or ongoing exposure to the causative agent (Steinman et al., 2014).

In Korea, foal diseases predominantly include neonatal isoerythrolysis (Kwon et al., 2011), diarrhea resulting from *Clostridium perfringens* type C (Park et al., 2019), and pneumonia caused by *Rhodococcus equi* (Song et al., 2019). Although numerous studies have investigated these diseases, this study represents the first exploration of *Lawsonia intracellularis*. Immunohistochemical procedures utilizing tissue or biopsy samples have proven effective in diagnosing EPE subsequent to the identification of specific antibodies against *Lawsonia intracellularis*. Although previous serological studies have been conducted using limited samples, it is deemed imperative to maintain continuous monitoring of domestically bred foals based on the insights gleaned from this study.

Author Contributions

All research protocols and animal experiments in this study were designed and conducted by DS Kim, A Irgashev, J Orozov and GJ Cho, who also contributed to data acquisition. GJ Cho further contributed to interpreting the experimental results and drafting the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology (NRF-2020R111A3067905).

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