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Seroprevalence and molecular detection of Lawsonia intracellularis from asymptomatic horses in Korea

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Short Communication

Seroprevalence and molecular detection of *Lawsonia intracellularis* from asymptomatic horses in Korea

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Abstract

The study was performed to investigate the prevalence pattern of *Lawsonia intracellularis* in horses in Korea via serology and molecular detection. Total of 138 horse sera and 112 fecal samples, in pairs with sera, were collected from five provinces across the country. The sera were analyzed using blocking enzyme-linked immunosorbent assay (bELISA) to detect *L. intracellularis*-specific antibodies and the fecal samples were evaluated by the real-time PCR assay to detect DNA of *L. intracellularis*. Results showed that 94% of the horses had antibodies and the younger the age, the higher the seropositivity; "<2 years" (92%), "2-5 years" (96%), "6-10 years" (94%), and ">10 years" (93%). DNA of *L. intracellularis* was identified in 18% of the horses and neither sex- nor breed-dependent difference was observed. Rather, like serology, the younger the age, the higher the positivity; "<2 years" (43%), "2-5 years" (25%), "6-10 years" (13%), ">10 years" (8%). The results indicated that the agent was prevalent across various age groups and once infected at a young age, it could persist for a long time in the groups, spreading via fecal-oral route. Although the research was conducted on clinically healthy horses, a considerable portion of horses were found to be subclinically infected. Therefore, clinicians as well as horse industry workers should be alert to the pathogen as another must-have test to establish diagnostic criteria for equine enteric diseases.

Keywords: equine proliferative enteropathy, horse, Korea, *Lawsonia intracellularis*, prevalence

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Introduction

Lawsonia intracellularis is the etiologic agent of a small intestinal disease called equine proliferative enteropathy (EPE). It has been increasingly diagnosed in horses and is now a significant problem in the husbandry. It commonly affects foals (3-7 months of age) (Bihr, 2003; Deprez et al., 2005; Lavoie et al., 2000; Pusterla et al., 2008), causing clinical signs of diarrhea, profound dullness, fever, rapid weight loss, colic, rough hair coat, and ventral edema due to severe hypoproteinemia (Bihr, 2003; Feary and Hassel, 2006; Frazer, 2008; Kimberly et al., 2007). *L. intracellularis* is a microaerophilic, obligate intracellular, curved or S-shaped, gram-negative bacterium found in the apical cytoplasm of infected enterocytes causing hyperplasia, which results in thickened small and sometimes large intestines (Lawson and Gebhart, 2000). In addition to horses, multiple host species have been considered to be susceptible to *L. intracellularis* including free-living animals, such as rabbits, rodents, foxes, wild pigs, deer, ferrets, ostriches, raccoon dogs, Korean water deer, non-human primates, domestic dogs and pigs (Dezorzova-Tomanova et al., 2006; Friedman et al., 2008; Hossain et al., 2016; Klimes et al., 2007; Lawson and Gebhart, 2000; Tomanova et al., 2003). The pathogen can persist and be shed to the environment via these various reservoirs (Lawson and Gebhart, 2000).

L. intracellularis-induced EPE has been reported in various regions of North and South America, Australia, and Europe (Deprez et al., 2005; Feary and Hassel, 2006; Wattanaphansak et al., 2010), but not in Asia. Despite the wide prevalence of the malady and the financial impact from associated losses, to our knowledge, the prevalence of *L. intracellularis* in the equine species has not been investigated in Korea. Instead, it is best described in swine, which is endemic.

Factors that predispose to EPE are unknown, but it has been suggested that several management factors, such as transportation, ration changes, antibiotic administration, or mixing, seem to be associated with onset of the disease (Lavoie et al., 2000; Winkelman, 1996). The spread of infection over horses might occur through the ingestion of feed or water contaminated with *L. intracellularis*-containing feces. Due to difficulties in culturing this obligate intracellular bacterium *in vitro*, diagnostic tools for detecting exposure to or infection with *L. intracellularis* have been based on serology, fecal PCR, and immunohistochemistry in tissue samples (Friedman et al., 2008; Guedes, 2004).

The present study was conducted to determine the prevalence of *L. intracellularis* in Korean horses through serology and fecal PCR because subjects to be investigated were clinically healthy live horses.

Materials and Methods

Blood and fecal samples: This study included horses reared throughout Korea, in which horse farms operate like cattle farms, with as many as 100 horses or as little as 3 to 20 horses. The total number of horses is approximately 30,000 in Korea as of 2015 (Lee et al., 2015) and there are reports on intermittent GI problems like diarrhea but not life-threatening diseases. The study was carried out with samples obtained from medical practice of the concept of health examination. Blood samples were collected from 138 healthy horses which did not present any abnormality by thorough clinical examination. The horses were raised in 5 different locations (Jeonbuk, 35°49′N and 127°09′E; Gyeonggi, 37°30′N and 127°15′E; Gyeongbuk, 35°87′N and 128°60′E; Busan, 35°18′N and 129°07′E; and Jeju, 33°49′N and 126°53′E) in Korea. Fecal samples were obtained from 112 of them from January to December 2014 (Fig. 1). Laboratory analyses were performed at the Veterinary Diagnostic Center of the College of Veterinary Medicine at Chonbuk National University, Korea.

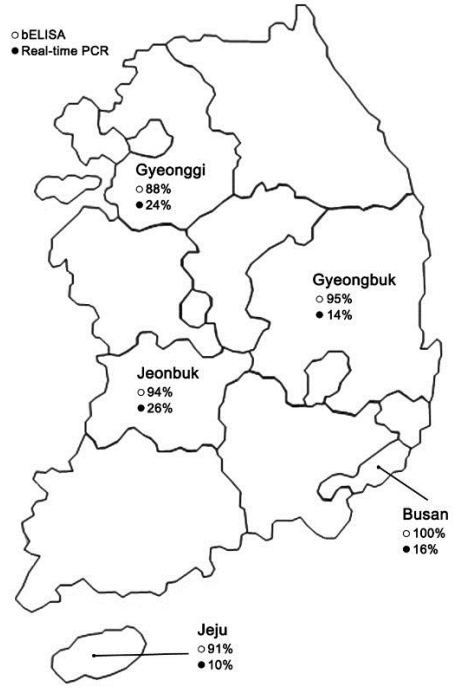

Figure 1 A map of South Korea showing the five different study regions where blood and fecal samples were collected from horses to detect *Lawsonia intracellularis*

The sera for serology were separated within 24 hours after blood collection at 4ºC and stored at -20ºC until tested for specific antibodies against *L. intracellularis* via bELISA analysis. The fecal samples for molecular detection were kept at 4ºC within 24 hours after collection and extraction of DNA was done according to the manufacturer's instructions using Exgene™ Stool DNA mini (GeneAll Biotechnology Ltd., Korea). Briefly, 1 ml of phosphate buffered saline (PBS) was added to 200 mg of freshly collected feces and mixed until thoroughly homogenized. After centrifugation, the supernatant was transferred to an Ezpass™ filter (GeneAll Biotechnology Ltd., Korea), centrifuged, added with 100 µL of the elution buffer and incubated for one minute at room temperature. Then, after another centrifugation, the filter column was washed and DNA elution was done with 30 µL of the elution buffer. The eluted DNA samples were stored at -80ºC until further analysis.

This study was approved by Institutional Committee of Graduate Studies and Research at Chonbuk National University, Jeonju, Korea (Approval no. CBNU 2017-0031).

Serology: Serology was applied to detect specific antibodies against *L. intracellularis* using a commercially available blocking ELISA (bELISA) kit (bioScreen Ileitis Antibody ELISA, Synbiotics Co., France) (Kranenburg et al., 2011; Hossain et al., 2016). Positive and negative control sera were supplied by the manufacturer. According to the manufacturer's instructions, the serum samples were placed in each well together with controls which were prepared from natural hosts as well as those provided by the manufacturer. Following incubation and wash steps, a monoclonal antibody to an *L. intracellularis* epitope conjugated with peroxidase was added to the wells. If a sample contained anti-*L. intracellularis* antibodies, the antigenic sites would be blocked to prevent binding of the monoclonal antibody conjugate. In these cases, color development was either very low or absent. If a sample did not contain anti-*L. intracellularis* antibodies, the antigen would be free to bind to the monoclonal antibody, resulting in intense color development. Optical density was measured at 450 nm in a microplate reader, and percent inhibition (PI) was calculated for positive controls and test samples relative to the negative controls. Serum samples with PI ≥30% were considered as positive while samples with PI <30% were regarded as negative.

Real-time PCR amplification and sequencing: The real-time PCR analysis was performed to detect the aspartate ammonia-lyase gene (*aspA*) of *L. intracellularis* as previously described by Wattanaphansak et al. (2010) with some modifications. Using the primers, Li-F (5′-GCTGTGGATTGGGAGAAATC-3′) and Li-R (5′-CAAGTTGACCAGCCTCTGC-3′), the reaction reagent consisted of 10 µL of PowerUp™ SYBR™ Green Master Mix (2X) (Applied Biosystems, CA, USA), 800 nM each of the Li-F and Li-R primers and 5 µL of the template DNA and was adjusted to a final volume of 20 µL with Rnase/Dnase free water. The amplification sequence was as follows: incubation with uracil-DNA glycosylase for 2 min at 50ºC, Dual-Lock™ DNA Polymerase activation for 2 min at 95ºC, 40 cycles of denaturation at 95ºC for 15 sec, annealing at 60ºC for 15 sec, and primer extension at 72ºC for 1 min. The DNA of *L. intracellularis* strain B3903 was employed as the positive control for the assay, which was extracted from the vaccine for swine use. To quantify the amount of DNA, *L. intracellularis* strain PHE/MN1-00 (ATCC PTA-3457) maintained in our laboratory was used. Ten-fold serial dilutions of the standard *L. intracellularis* DNA were added into each run to generate the standard curve. All samples, negative controls, and the standard *L. intracellularis* DNA were tested in the triplicate real-time PCR wells in the same run. All reactions were performed on a 7500 Fast real-time PCR system (Applied Biosystems, CA, USA) and data were analyzed using the 7500 Software v.2.0.5 (Applied Biosystems, CA, USA). For sequence analysis, the PCR product was sent to the company, Macrogen Inc (Seoul, Korea). To determine specificity of the selected primers, PCR assays were conducted with *L. intracellularis* PHE/MN1-00, and four non-*L. intracellularis* bacteria: *Clostridium perfringens* (ATCC 13124), *Cl. difficile* (ATCC 9689), *Salmonella enterica serovar typhimurium* (ATCC 140280) and *Rhodococcus equi* [Korean Collection for Type Cultures (KCTC) 1298].

Data analysis: Summary statistics were calculated to assess overall quality of the data. Chi-square analysis was used to compare each control sample, to determine the significance of differences in the prevalence among regions and among animal species. Pearson's correlation analysis was used to compare results from the bELISA and real-time PCR assays. *P*-values of <0.05 were considered to be significant using SPSS 17.0 (SPSS Inc., USA).

Results and Discussion

One hundred and thirty (94%) of the 138 sera had antibodies against *L. intracellularis* and twenty (18%) of the 112 fecal samples were positive for the antigen (Table 1). In the serology, geographically, Busan area had the highest prevalence of antibodies to *L. intracellularis* (100%, $\chi^2 = 7.246$, df = 4, $P < 0.05$), followed by Gyeongbuk (95%), Jeonbuk (94%), Jeju (91%), and Gyeonggi (88%). By age, almost all age groups were found to have positive antibody titers against *L. intracellularis* ranging from 93% to 96% ($\chi^2 = 0.270$, df = 3, $P = 0.966$). By sex, geldings had highest antibody titers against *L. intracellularis* (98%, $\chi^2 = 8.585$, df = 2, $P < 0.05$), followed by males (95%), and females (91%). By breed, all of the tested Juju horses had antibodies to *L. intracellularis* (100%), followed by Thoroughbreds (95%), other breeds (86%), and Hallas (83%, $\chi^2 = 2.531$, df = 3, $P = 0.470$).

For the real-time PCR assays, geographically, a positive rate was found throughout Korea; Jeonbuk had the highest prevalence rate (26%), followed by Gyeonggi (24%), Busan (16%), Gyeongbuk (14%) and Jeju (10%). The prevalence rate of Jeonbuk and Gyeonggi was significantly higher than that of the other areas ($\chi^2 = 7.891$, df = 4, $P < 0.05$). By age, the "<2 years" group was significantly higher in the real-time

PCR assay than the other age groups (43%, $\chi^2 = 9.027$, df = 3, $P < 0.05$) including "2-5 years" (25%), "6-10 years" (13%), and ">10 years" groups (8%). By sex, the prevalence rate was not much different from 17% to 18% ($\chi^2 = 0.035$, df = 2, $P = 0.983$). By breed, positivity was detected most often in other breeds (25%), followed by Thoroughbreds (18%), Hallas (17%), and Jeju horses (13%). No statistically significant differences were found ($\chi^2 = 0.441$, df = 3, $P = 0.932$). All fecal samples examined were paired with the sera samples and the Pearson's correlation analysis suggested that the results of bELISA and real-time PCR assays significantly correlated ($r = 0.729$, $P < 0.001$).

Table 1 Prevalence of *Lawsonia intracellularis* in horses according to various variables: regions of Korea, age, sex, and breed, tested by blocking enzyme-linked immunosorbent assay (bELISA) and real-time PCR from January to December 2014

Variables	bELISA		Real-time PCR	
	Tested (N)	Positive (%)	Tested (N)	Positive (%)
Areas				
Jeonbuk	53	50 (94)	27	7 (26)*
Gyeonggi	17	15 (88)	17	4 (24)*
Gyeongbuk	22	21 (95)	22	3 (14)
Busan	25	25 (100)*	25	4 (16)
Jeju	21	19 (91)	21	2 (10)
Age				
<2 years	14	13 (93)	14	6 (43)*
2-5 years	26	25 (96)	20	5 (25)
6-10 years	68	64 (94)	54	7 (13)
>10 years	30	28 (93)	24	2 (8)
Sex				
Male	21	20 (95)	18	3 (17)
Female	67	61 (91)	51	9 (18)
Gelding	50	49 (98)*	43	8 (19)
Breed				
Thoroughbred	116	110 (95)	90	16 (18)
Jeju horse	8	8 (100)*	8	1 (13)
Halla	6	5 (83)	6	1 (17)
Others#	8	7 (88)	8	2 (25)
Total	138	130 (94)	112	20 (18)

#Others = American appaloosa; American Quarter Horse; Percheron cross; Mongolian pony; Polish Arab
*$P < 0.05$

The present study found that more than 90% (an average of 94.16%) of the horses were seropositive for *L. intracellularis* in Korea, which is consistent with the results of previous studies; adult horses are commonly exposed to *L. intracellularis* (Feary et al., 2007). Thus, the younger the animals (less than 2 years), the lower the prevalence (92%). Other investigations showed that older horses were detected more often to be positive (99%) than yearlings (89%) (Kranenburg et al., 2011). It makes it possible to assume that horses have been constantly exposed to *L. intracellularis* (Guimarães-Ladeira et al., 2009) since they were young. Similar results postulated that the seroprevalence against *L. intracellularis* in young horses was 60.6%, compared to 87.1% in horses over one year of age (Klimes et al., 2007) and 14% of foals had a positive antibody titer before weaning, but 23% had a positive titer after weaning. Foals seropositive for *L. intracellularis* have been shown to remain seropositive for more than 6 months (Wattanaphansak et al., 2010). Several previous studies examined the persistence of antibodies against *L. intracellularis* in pigs; naturally infected pigs seemed to be boosted by *L. intracellularis* present in the herd (Boesen et al., 2005), pigs remained seropositive for up to 13 weeks after exposure (Guedes and Gebhart, 2003), and the positive antibodies were persistently detected in pigs after 4 months (Boesen et al., 2005; Deprez et al., 2005). In a dog study, positive antibodies to *L. intracellularis* were detected over a period of 291 days (Tomanova et al., 2003). Because the bELISA kit is designed for swine use, it was evaluated with the reactivity between species, the pig serum and horse serum as previously described (Hossain et al., 2016).

In this study, most of the seropositive horses did not secrete the bacteria, which indicates that horses in good condition might not actively contribute to increase the burden of *L. intracellularis* in the environment. This is in sharp contrast to the case of pigs which have been shown to shed *L. intracellularis* early in their lives, and substantially to cause build-up of the bacteria in the environment for a long duration (Guedes et al., 2002). Interestingly, a 3-month-old foal was positive in the real-time PCR assay as well as serology, and remained clinically healthy until the end of the study. These results were consistent with studies conducted elsewhere (Pusterla et al., 2009). Most reported cases of *L. intracellularis* infections were young animals aged less than one year (Pusterla et al., 2009) as the maternal antibodies waned, although Guimarães-Ladeira et al. (2009) reported horses aged between 13 and 16 months shedding the bacteria in feces. The management changes could cause stress and predispose this age group to be affected by the disease (Frazer, 2008). An amount of literature indicated that clinically normal horses could be positive for *L. intracellularis* by the PCR assay, serology, or both (Frazer, 2008; Guimarães-Ladeira et al., 2009).

In other species, porcine proliferative enteropathy (PPE) has been actively investigated and it was reported that 44% to 69% of studied pigs were positive in serology (Lee et al., 2001). Also, Yeh (2014) reported 23.04% of clinically healthy wild boars harboring antibodies to *L. intracellularis* in Korea. However, in molecular detection assay, 7.9% of studied pigs resulted in being positive for DNA of *L. intracellularis* by PCR (Chu et al., 2010), although Park et al. (2015) reported 80.3% of *L. intracellularis* DNA-positive pigs in the study group by loop-mediated isothermal amplification assay which was developed with high sensitivity for field diagnostic use. When animals are seropositive and PCR-negative, either they have been previously exposed to *L. intracellularis* and are no longer shedding the organism, or detection of fecal shedding is limited by the low sensitivity of the PCR technique in fecal samples (Guedes, 2004).

In addition to pigs, a wide range of animal species were detected to be positive for *L. intracellularis*, including hamsters, guinea pigs, dogs, lambs, calves, ferrets, foxes, deer, rabbits, rats, mice, ratites, wild boars, wolves, giraffes, hedgehogs, Korean water deer, raccoon dogs, and primates (Dezorzova-Tomanova et al., 2006; Friedman et al., 2008; Klimes et al., 2007; Lee et al., 2001; Hossain et al., 2016; Pusterla et al., 2008). The broad range of multiple host species has raised questions whether natural cross-species transmission might be possible. Cross-species transmission has already been demonstrated experimentally among pigs, horses, hamsters and mice, and the disease in non-human primates has recently been reported (Sampieri et al., 2013; Vannucci et al., 2012). Further studies using validated methods for cross-species including human transmission would be required to better define molecular and/or functional features of this sophisticated pathogen. The results of the present study indicate that the agent is prevalent, and therefore clinicians as well as horse industry workers should be alert to the pathogen as another possible cause of enteric diseases in order to provide better antemortem diagnoses.

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References

Bihr TP 2003. Protein-losing enteropathy caused by *Lawsonia intracellularis* in a weanling foal. Can Vet J. 44(1):65-66.

Boesen HT, Jensen TK, Moller K, Nielsen LH and Jungersen G 2005. Evaluation of a novel enzyme-linked immunosorbent assay for serological diagnosis of porcine proliferative enteropathy. Vet Microbiol. 109(1-2):105-112.

Chu JQ, Hu XM, Kim MC, Park CS and Jun MH 2010. Detection and genetic characterization of *Lawsonia intracellularis* from swine in Korea. Korean J Vet Serv. 33(3):223-231.

Cooper DM and Gebhart CJ 1998. Comparative aspects of proliferative enteritis. J Am Vet Med Assoc. 212(9):1446-1451.

Dauvillier J, Picandet V, Harel J, Gottschalk M, Desrosiers R, jean D and Lavoie JP 2006. Diagnostic and epidemiological features of *Lawsonia intracellularis* enteropathy in 2 foals. Can Vet J 47(7):689-691.

Deprez P, Chiers K, Gebhart CJ, Ducatelle R, Lefere L, Vanschandevijl K and van Loon G 2005. *Lawsonia intracellularis* infection in a 12-month-old colt in Belgium. Vet Rec. 157(24):774-776.

Dezorzova-Tomanova K, Smola J, Trcka I, Lamka J and Pavlik I 2006. Detection of *Lawsonia intracellularis* in Wild Boar and Fallow Deer Bred in One Game Enclosure in the Czech Republic. J Vet Med B Infect Dis Vet Public Health, 53(1):42-44.

Feary DJ, Gebhart CJ and Pusterla N 2007. *Lawsonia intracellularis* proliferative enteropathy in a foal. Schweiz Arch Tierheilkd, 149(3):129-133.

Feary DJ and Hassel DM 2006. Enteritis and Colitis in Horses. Vet Clin North Am Equine Pract, 22(2):437-479.

Frazer ML 2008. *Lawsonia intracellularis* infection in horses: 2005-2007. J Vet Intern Med, 22(5):1243-1248.

Friedman M, Bednar V, Klimes J, Smola J, Mrlík V and Literak I 2008. *Lawsonia intracellularis* in rodents from pig farms with the occurrence of porcine proliferative enteropathy. Lett Appl Microbiol, 47(2):117-121.

Guedes R 2004. Update on epidemiology and diagnosis of porcine proliferative enteropathy. J Swine Health Prod, 12(3): 134-138.

Guedes RM, Gebhart CJ, Armbruster GA and Roggow BD 2002. Serologic follow-up of a repopulated swine herd after an outbreak of proliferative hemorrhagic enteropathy. Can J Vet Res, 66(4):258-263.

Guedes RM and Gebhart CJ 2003. Onset and duration of fecal shedding, cell-mediated and humoral immune responses in pigs after challenge with a pathogenic or attenuated vaccine strain of *Lawsonia intracellularis*. Vet Microbiol, 91(2-3):135-145.

Guimarães-Ladeira CV, Palhares MS, Oliveira JS, Ramirez MA and Guedes RM 2009. Faecal shedding and serological cross-sectional study of *Lawsonia intracellularis* in horses in the state of Minas Gerais, Brazil. Equine Vet J, 41(6):593-596.

Hossain MM, Oh Y and Cho HS 2016. Prevalence of antibody to and DNA of *Lawsonia intracellularis* in samples from wild animals in Korea. J Wildl Dis, 52(4):803-808.

Jacobson M, Rasback T, Floistrup H, Benz M, Braun-Fahrlander C, Riedler J, Schram-Bijkerk D and Fellstrom C 2007. Survey on the occurrence of *Brachyspira* species and *Lawsonia intracellularis* in children living on pig farms. Epidemiol Infect, 135(6):1043-1045.

Kranenburg LC, van Ree HE, Calis AN, de Pater M, Buter GJ, van Maanen C and Sloet van Oldruitenborgh-Oosterbaarn MM 2011. The seroprevalence of *Lawsonia*

intracellularis in horses in The Netherlands. Tijdschr Diergeneeskd, 136(4):237-243.

Kimberly M, McGurrin J, Modest V, Arroyo LG and Baird JD 2007. An outbreak of *Lawsonia intracellularis* infection in a standardbred herd in Ontario. Can Vet J, 48(9):927-930.

Klimes J, Dezorzova K, Smola J and Husnik R 2007. Prevalence of antibodies against *Lawsonia intracellularis* in dogs with and without gastrointestinal disease. Vet Med Czech, 52(11):502-506.

Lavoie JP, Drolet R, Parsons D, Leguillette R, Sauvageau R, Shapiro J, Houle L, Hallé G and Gebhart CJ 2000. Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinemia in foals on three breeding farms in Canada. Equine Vet J, 32(5):418-425.

Lawson GH and Gebhart CJ 2000. Proliferative enteropathy. J Comp Pathol, 122(2-3):77-100.

Lee SW, Kim TJ, Park SY, Song CS, Chang HK, Yeh JK, Park HI and Lee JB 2001. Prevalence of porcine proliferative enteropathy and its control with tylosin in Korea. J Vet Sci, 2(3):209-212.

Lee SH, Kim KT, Yun SH, Choi E, Lee GH, Park YS, Cho KH, Yi S, Kwon OD, Kim TH, Kwak D 2015. Serological and molecular detection of Anaplasma phagocytophilum in horses reared in Korea. Vet Med, 60(10): 533-538.

Park BY, Shim KS, Kim WI, Hossain MM, Kim BS, Kwon JK, Park CK, Cho SJ, Jo IH and Cho HS 2015. Rapid and sensitive detection of *Lawsonia intracellularis* in pigs by real-time loop-mediated isothermal amplification. Acta Veterinaria-Beograd, 65(1):20-29.

Pusterla N, Jackson R, Wilson R, Collier J, Mapes S and Gebhart CJ 2009. Temporal detection of *Lawsonia intracellularis* using serology and real-time PCR in Thoroughbred horses residing on a farm endemic for equine proliferative enteropathy. Vet Microbiol, 136(1-2):173-176.

Pusterla N, Mapes S, Rejmanek D and Gebhart C 2008. Detection of *Lawsonia intracellularis* by real-time PCR in the feces of free-living animals from equine farms with documented occurrence of equine proliferative enteropathy. J Wildl Dis, 44(4):992-998.

Sampieri F, Vannucci FA, Allen AL, Pusterla N, Antonopoulos AJ, Ball KR, Thompson J, Dowling PM, Hamilton DL, Gebhart CJ. 2013. Species-specificity of equine and porcine *Lawsonia intracellularis* isolates in laboratory animals. Can J Vet Res, 77:261-272.

Tomanova K, Klimes J, Smola J and Husnik R 2003. Detection of *Lawsonia intracellularis* in a dog with inflammatory bowel disease using nested PCR and serology. Vet Med Czech, 48(4):108-112.

Vannucci FA, Pusterla N, Mapes SM, Gebhart C. 2012. Evidence of host adaptation in *Lawsonia intracellularis* infections. Vet Res, 43:53.

Wattanaphansak S, Gebhart CJ, Anderson JM and Singer RS 2010. Development of a polymerase chain reaction assay for quantification of *Lawsonia intracellularis*. J Vet Diagn Invest, 22(4):598-602.

Winkelman NL 1996. Ileitis: an update. Comp cont Educ pract Vet, 18: s19-s25.

Yeh JY 2014. Seroprevalence of porcine proliferative enteropathy among wild boars in the Republic of Korea. BMC Vet Res, 10:5.

บทคัดย่อ

ความชุกทางซีรัมวิทยาและการตรวจหาโมเลกุลของเชื้อ *Lawsonia intracellularis* จากม้าที่ไม่แสดงอาการในประเทศเกาหลี

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การศึกษาครั้งนี้เป็นการตรวจหาความชุกทางซีรัมวิทยาและตรวจหาเชื้อในระดับโมเลกุล ของเชื้อ *Lawsonia intracellularis* ในม้า โดยเก็บตัวอย่างซีรัมจำนวน 138 ตัวอย่างและตัวอย่างอุจจาระจำนวน 112 ตัวอย่าง (เก็บตัวอย่างเป็นคู่กับซีรั่ม) ใน 5 จังหวัดทั่วประเทศเกาหลี โดยตัวอย่างซีรั่มจะตรวจหาแอนติบอดีที่เฉพาะเจาะจงต่อเชื้อ *L. intracellularis* ด้วยวิธี bELISA และตัวอย่างอุจจาระจะตรวจหาดีเอ็นเอของเชื้อ *L. intracellularis* ด้วยวิธี realtime-PCR ผลการศึกษาพบว่า 94% ของม้ามีแอนติบอดีต่อเชื้อ และพบว่าม้าอายุน้อยจะมีผลบวกมาก ได้แก่ อายุ 2-5 ปี (96%) อายุ 6-10 ปี (94%) และ อายุ > 10 ปี (93%) ส่วนผลการตรวจหาเชื้อในระดับโมเลกุล พบว่า 18% ของม้าพบดีเอ็นเอของเชื้อ และไม่พบความแตกต่างระหว่างเพศหรือสายพันธุ์ และพบว่าสัตว์ที่อายุน้อยจะมีผลบวกมากเช่นกัน ได้แก่ อายุ 2-5 ปี (25%) อายุ 6-10 ปี (13%) และ อายุ > 10 ปี (8%) ผลการศึกษาแสดงให้เห็นว่า เชื้อมีการแพร่กระจายในสัตว์ทุกกลุ่มอายุ และเมื่อสัตว์อายุน้อยติดเชื้อแล้วอาจอยู่ได้นาน โดยจะแพร่กระจายผ่านทางอุจจาระและปาก การวิจัยครั้งนี้ได้ศึกษาในม้าสุขภาพดี และพบการติดเชื้อแบบไม่แสดงอาการ ดังนั้นสัตวแพทย์และผู้ดูแลม้าควรให้ความสำคัญ และควรมีการสร้างเกณฑ์การวินิจฉัยโรคต่อไป

คำสำคัญ: enteropathy proliferative ม้า เกาหลี *Lawsonia intracellularis* ความชุก

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