Evidence of Lawsonia intracellularis Infections in Domestic Cats with Intestinal Disorder: A Retrospective Serological Study

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ABSTRACT

Basic information regarding *Lawsonia intracellularis* is still limited despite worldwide distribution, relatively high prevalence, economic impacts to the swine industry, and recent identification of additional susceptible animal hosts. Although diseases closely resembling proliferative enteropathy and caused by *L intracellularis* have been described in case reports for a wide range of host species including: rodents, deer, emus, non-human primates, and rhesus macaques, in most cases, the origin of *L.intracellularis* is unknown. The current study investigated the *Lawsonia intracellularis*-specific antibody prevalence in domestic cats (*Felis catus*) visiting for treatment of intestinal disorders to animal clinics in South Korea. In this study, 11 of 235 cats visiting for treatment of intestinal disorders were found to be antibody positive to *L. intracellularis* and TP calculated based on accuracy of the test was 5.2% (95% confidence intervals: 3.8-6.6%). Antibody prevalence of Gyeonggi province was slightly higher compared to that of Seoul province. Interestingly, most of the seropositive cats in the current study lived in urban areas and did not have any history of contact with pigs or horses. These results imply that *L intracellularis* may play a role in gastrointestinal diseases of South Korean cat populations. Additionally, results of this study suggest the importance of cats in the transmission of *L intracellularis* among domestic animal species which may serve as foundation for future research.

INTRODUCTION

Proliferative enteropathy (PE) is an infectious intestinal disease characterized by thickening of the distal small and proximal large intestinal mucosa as a result of enterocyte proliferation associated with the presence of an intracellular bacterium, *Lawsonia intracellularis*.[1] Although diseases closely resembling PE and caused by *L intracellularis* have been described in case reports for a wide range of host species, including ro-

dents, deer, emus, non-human primates, and rhesus macaques,[2-7] in most cases, the origin of *L. intracellularis* is unknown. Basic information regarding *L intracellular* is still limited despite worldwide distribution, relatively high prevalence, economic impacts to the swine industry, and recent identification of additional susceptible animal hosts. Isolation and in vitro cultivation of *L. intracellularis* has proven to be difficult, which has contributed to limited research progress. Although the epidemiology of porcine and equine PE is now better understood, interspecies transmission and infection status of *L intracellularis* in other species have not been sufficiently explored. For instance, no data on the serological prevalence of PE in animal species other than swine and horse have been published to date.[3,8-10]

Herbst et al and Pusterla et al investigated the presence of *L intracellular* DNA in domestic cats (*Felis catus*; n = 50 and n = 14, respectively), but did not found any evidence of this bacterium in feces.[11,12] No other study has been reported on association of *L intracellular* in the Felidae species. While initially recognized as the causative agent of PE in pigs, *L intracellularis* is now viewed as an emerging cause of intestinal health problems involving enteritis, hyperplasia, hemorrhage, or protein-losing enteropathy in a wide range of mammalian species. The aim of this study was to collect preliminary data on the seroprevalence of *L intracellular* antibodies in domestic cats in South Korea. We undertook our investigation for *L intracellularis* antibodies using an immunoperoxidase monolayer assay (IPMA).

MATERIALS AND METHODS

Serum Samples

Sera was collected from 235 domestic cats visiting for treatment of intestinal disorders (eg, acute or chronic diarrhea, abdominal pain, weight loss, abdominal masses, and severe nutrient deprivation) to nine animal clinics in Seoul and Gyeonggi provinces of South Korea from 2009 to 2011. Some samples were collected from clinically healthy cats post treatement (n = 36). Serum samples were stored at -20 °C prior to analysis.

Immunoperoxidase Monolayer Assay (IPMA)

IPMA was carried out to investigate *L intracellularis* antibodies in domestic cats in South Korea. The pathogenic isolate, PHE/KK421 (Korean Collection for Type Cultures 10686BP, Daejeon, South Korea), were used for the infection of murine fibroblast-like McCoy cells (American Type Culture Collection CRL 1696, VA, USA). Cultivation of *L intracellularis* and serology using IPMA technique were performed as described previously,[13-15] which was modified by using anti-cat IgG antibodies instead of anti-porcine IgG antibodies. Briefly, *L intracellularis* culture plate was incubated with sera diluted at 1:30 in phosphate-buffered saline (PBS) for 30 min at 37 °C, and washed five times with PBS, pH 7.2. Peroxidase-labeled goat anti-cat IgG was diluted 1:500 (KPL, MD, USA) in 2% bovine serum albumin (BSA) and 0.08% Tween80 in PBS, and then added at a concentration of 50 μL/well. The plate was incubated for 45 min at 37 °C. The plate was washed again, and chromogen (3-amino-9-ethyl-carbazole, Dako Corporation, CA, USA) solution was added to each well. The plate was then incubated at room temperature for 20 min. The plate was washed with distilled water 3 times, allowed to dry, and examined using a BX50 microscope (Olympus, Tokyo, Japan). Positive samples had red-labeled bacteria in the cytoplasm of infected McCoy cells and also extracellularly.

Statistical Analysis

True prevalence (TP) was estimated, as described by Marchevsky et al[16-19] using published test sensitivity and specificity of 100% and 90%, respectively.[15] The formula used to determine TP was: TP = (apparent prevalence+specificity-1)/(sensitivity+specificity-1). Statistical analyses were performed with the NCSS 2007 Statistical Software package (NCSS Statistical System for Windows, Kaysville, UT, USA) and the program 'Survey Toolbox Version 1.04'.[20]

Table 1. *Seroprevalence and 95% confidence intervals (CI) for Lawsonia intracellularis in cats visiting for treatment of intestinal disorder in South Korea from 2009 to 2011.*

Locality	No. of tested sample	No. of positive sample	AP %	TP % (95% CI)
Seoul	91	3	3.3	3.7 (1.7-5.6)
Gyeonggi	144	8	5.6	6.2 (4.2-8.2)
Total	235	11	4.7	5.2 (3.8-6.6)

Abbreviation: AP, apparent prevalence calculated from calculated directly from the sample; TP, true prevalence calculated based on accuracy of the test.

RESULTS

In our study, 11 of 235 cats visiting for treatment of intestinal disorders were found to be antibody positive to *L intracellularis* and TP calculated based on accuracy of the test was 5.2% (95% confidence intervals: 3.8-6.6%; Table 1). Antibody prevalence of Gyeonggi province was slightly higher compared to that of the Seoul province. Interestingly, most of the seropositive cats in the current study lived in urban areas and did not have any history of contact with pigs or horses. Therefore, it remains difficult to explain when and where cats were exposed to *L intracellularis*. No further inference was obtained after reviewing full medical histories of seropositive cats.

DISCUSSION

In previous study, Herbst et al did not find evidence of *L intracellularis* DNA in fecal samples of cats.[11] Although the antibody prevalence to *L intracellularis* in cats was low in this study, this serological evidence suggests that some cats were probably exposed to and infected with *L intracellularis*. However, methodological improvements in future studies may allow for improved epidemiological inference.

Cats were sampled on a single occasion in the current study which reflected past exposure to *L intracellularis*. However, it could not be determined whether the antibody positive cats were in a state of ongoing infection or recovery at the time of sampling. Paired sera for assessing a change in antibody titer between two sampling occasions and/or bacterial examination on intestinal tissues or feces could provide data on infection duration and timing of seroconversion.

Our results imply that *L intracellularis* may play a role in gastrointestinal diseases of South Korean cat populations. Additionally, results of this study suggest the importance of cats in the transmission of *L intracellularis* among domestic animal species, which may serve as foundation for future research. Further investigations of anti-*L intracellularis* antibodies in cat populations throughout South Korea should be carried out to better understand the role of companion animals in disease epidemiology.

In conclusion, *L intracellularis* could be a causative agent of some intestinal disorders in domestic cats from South Korea and/or may have a role in the exacerbation of signs. Therefore, screening of domestic cats with intestinal disorders for the presence of *L intracellularis* bacteria and/or antibodies is recommended. Veterinarians may also consider use of anti- *L. intracellularis* drugs in cats infected by this bacterium.

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