pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 35(5) : 175-177 (2018)
http://dx.doi.org/10.17555/jvc.2018.10.35.5.175

Journal of Veterinary Clinics
www.ksvc.or.kr

PCR-based Prevalence of Feline Vector-borne Pathogens in Yangju and Gwacheon Cities, South Korea

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(Received: August 08, 2018 / Accepted: September 11, 2018)

Abstract : The objective of this polymerase chain reaction (PCR)-based research was to determine the prevalence of vector-borne pathogens in stray cats in Yangju and Gwacheon cities, South Korea. Total 50 stray cats were sampled for this PCR-based survey; 33 samples and 17 samples were collected from Yangju and Gwacheon cities, respectively. Total positive presence rates were 6%, 6% and 24% for hemotropic mycoplasmas, *Rickettsia* spp. and *Babesia* spp., respectively in this study. *Babesia* spp. was the predominant pathogen present in the stray cats of both cities followed by hemotropic mycoplasmas and *Rickettsia* spp. It is recommended that a large-scale study of the prevalence of infectious agents among stray cats should be undertaken in all regions of South Korea.

Key words **:** prevalence, blood-borne disease, PCR, stray cat.

Introduction

Many vector-borne pathogens in cats are clinically important. Vector-borne diseases are included such genera as *Babesia* spp, hemotropic mycoplasmas and *Rickettsia* spp. (15). Pathogen transmittable vectors of such pathogens include arthropods such as ticks, fleas and mosquitoes (8). Recently, feline vector-borne diseases have become clinically and epidemiologically important as they have a wide geographic distribution and spread quickly due to climate change and human behavioral factors such as traveling (2,12).

Rickettsia spp. are infecting a wide variety of mammals, but cause disease in very few of them. Clinical signs of *Rickettsia* spp. infection are usually observed soon after tick infestation and are mostly non-specific, consisting of fever, anorexia, lethargy, and joint pain (9).

Feline hemotropic mycoplasmas are small epicellular parasites that adhere to the erythrocytes of infected animals and cause feline infectious anemia (13) Clinically hemotropic mycoplasmas are identified by undertaking cytological examination or serologic tests but, these methods have been low sensitivity and specificity, frequently resulting in false negative results (13).

Feline babesiosis is a tick-borne protozoan disease that affects domestic and wild animal, as well as humans, worldwide. While babesiosis has been observed in dogs around the world, it is rarely observed in cats (5).

The prevalence of various contagious feline diseases have not yet been reported in Yangju and Gwacheon cities, South Korea. The objective of this study was to determine the prevalence of vector-borne pathogens among stray cats in two cities by using a polymerase chain reaction (PCR).

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Materials and Methods

50 stray cats were sampled; 33 samples were collected from Yangju city and 17 samples were collected from Gwacheon city. Blood was collected from each cat via the jugular vein and all samples were stored at −80°C.

The blood samples were used for the extraction of nucleic acid for the detection of blood-borne pathogens. For extraction of nucleic acid, POBGEN™ (KogeneBiotech, Seoul, South Korea) PCR kits were used. A total nucleic acid purification kit was used to extract both DNA and RNA, and the nucleic acid extraction process included the following; Initial, transfer of 140 μL of the liquid sample into nuclease-free microcentrifuge tubes followed by the addition of 10 μL proteinase K solution (20 mg/mL) and 300 μL buffer LB1. After which the mixture was included at incubate at 56°C for 20 min for digestion followed by the addition of 250 μL absolute ethanol. After vortexing, approximately 700 μL of the sample were transferred to a spin column placed in a 2 mL collection tube and the lid was gently closed, followed by and centrifuging for 15 s at 8,000 × *g*. The flow-through was discarded and 600 μL buffer WB1 was added to the spin column. The lid was then gently closed, and the sample centrifuged for 15 s at ≥ 8,000 × *g* to wash the column. Once again, the flow-through was discarded and 500 μL of buffer WB2 was added to the spin column and the lid was closed gently followed by centrifuging for 15 s at ≥ 8,000 × *g* to wash the column.

As above, the flow-through was discarded. Those steps were repeated twice. Next, the spin column was placed in a 2 mL collection tube and centrifuged at full speed for 1min and the flow-through discarded with the collection tube. After that, the spin column was placed in a new 1.5 mL collection tube and 50 μL of nuclease free water added directly to the spin column membrane, the lid closed gently, and the tube

Table 1. Prevalence of vector-borne pathogens of stray cat in Yangju and Gwacheon cities

City		Hemotropic mycoplasmas	Rickettsia spp.	Babesia spp.
Yangju	Number of positive	3/33	3/33	9/33
	Positive rate	9%	9%	27%
Gwacheon	Number of positive	0/17	0/17	3/17
	Positive rate	0%	0%	17%
Total	Number of positive	3/50	3/50	12/50
	Positive rate	6%	6%	24%

left to stand for 5 min then centrifuged for 1 min at ≥ 8,000 × *g* to elute the nucleic acids.

We selected ten pathogens; *Anaplasma* spp*, Erlichia* spp, *Babesia* spp*,* hemotropic mycoplasmas and *Rickettsia* spp.

We performed real-time PCR methods to detect the pathogens. The nucleic acid extracted from the feral cat blood samples was used as a template to detect the genes associated with each pathogens. After adding the nucleic acid extracted from blood into the master mix for the real-time PCR in a 20 uL reaction volume, which included pathogen specific primers and a probe, one step quantitative PCR was performed for the real-time amplicon detection using the following thermal conditions; 50°C for 10min to perform reverse transcription and genetic amplification followed by fluorescence at 95°C 10 sec and 60°C 30 sec for a total of 45 cycles.

Results

Anaplasma and *Erlichia* presence were all negative in Yangju city. However, 3 samples (9%) had positive results for hemotropic mycoplasmas and *Rickettsia* spp., while 9 samples (27%) showed a positive result for *Babesia* spp. (Table 1). Hemotropic mycoplasmas*, Rickettsia, Anaplasma* and *Erhlichia* pathogens were not detected in Gwacheon city. However, 3 samples (17%) had a positive result for *Babesia* spp. (Table 1).

The total positive rates for all sampled cats were 6%, 6% and 24% for hemotropic mycoplasmas*, Rickettsia* spp. and *Babesia* spp., respectively (Table 1). *Babesia* spp. had a higher presence than hemotropic mycoplasmas*, Rickettsia* spp. in the stray cats of both cities.

Discussion

In this study, we assessed the prevalence of vector-borne pathogens in 50 stray cats of Yangju (33 cats) and Gwacheon (17cats) cities. In the current study, *Anaplasma* and *Erhlichia* were not detected in either Yangju and Gwacheon cities, whereas, hemotrotic mycoplasmas, *Rickettsia* spp. and *Babesia* spp. tested positive in 9%, 9% and 27%, respectively, in Yangju city. In contrast, only *Babesia* spp. (17%) were detected in Gwacheon city. The prevalence of hemotropic mycoplasmas, *Rickettsia* spp. and *Babesia* spp. pathogens in Yangju city were higher than in Gwacheon city. *Babesia* spp. was the dominant infective pathogen detected in stray cats of both Yangju and Gwacheon cities followed by hemotropic mycoplasmas and *Rickettsia* spp.

Babesiosis is a well-known tick-borne zoonosis disease worldwide but, is rare in cats (5). *Babesia* spends the majority of its life cycle within the erythrocyte of the definitive host, resulting in hemolysis, with or without systemic complication (1). The genus *Babesia,* belongs to the *Piroplasmida* order is an intracellular parasites. *Babesia felis* can cause severe anemia in cats, but in other species, it is less pathogenic and rarely produces clinical signs (5). Ticks are infected by ingesting merozoites during feeding, and sporozoite form by replication of the parasite within their salivary cells (1). Diagnosis tools for *Babesia* infection in casts include blood smear and PCR-based methods (5). Treatment of babesiosis include administration of antiprotozoal drugs, but tick control is the best way to prevent infection (5). South Africa area with a high prevalence of feline babesiosis, but only in coastal regions (10). *B. felis*, originally identified in wild cats in Sudan, was subsequently found to cause clinical disease in domestic cats (10). Most other *Babesia* spp., such as *Babesia cati* found in India, are less pathogenic, while *Babesia leo,* common in lions of South Africa and Swaziland, is genetically similar to *B. felis* (3).

Babesia microti is an infectious agent detected in more than 300 human *Babesia* cases since 1969 (6). Its presence was also observed in 6 cats from southern Italy (11). Since domestic cats have a close relationship with humans, they are a potential source of *Babesia* infection; a previous report on the prevalence of *B. microti* showed that two of 260 feline samples (0.8%) were positive. For *B. microti*. The infection rate of *B. microti* in *Haemaphysalis longicornis* ticks obtained from cats and dogs was studied in Japan, and *B. microti* presence was positive in 13 out of 1,341 samples (7).

More specific classification of *Babesia* spp. was not carried out in the present study; thus, it is not possible to compare directly the results previously conducted research with those for Yangju (27%) and Gwacheon (17%) cities. Until now, report on the overall prevalence of *Babesia* in feral cat populations are scarce, especially in South Korea. The comparatively high prevalence observed in Yangju and Gwacheon cities emphasizes that detection of *Babesia* using PCR-based method is important in anemic feline patients.

In the current study, 3 samples (9%) were *Mycoplasma* positive in stray cats of Yangju city. In contrast, in Southern Brazil, among 369 domestic cats, a total of 79 samples (21.4%) were *Mycoplasma* positive (14), which is higher than that of the current study. In our study, we collected sample from stray cats, whereas, the samples were from domestic cats in Brazil (14). Notably, a positive rate for *Haemoplasma* presence (7.8%) in 116 shelter cats from the Barcelona area in Spain was similar to our Hemotropic mycoplasma result (12).

Previously, similar research was conducted in South Korea and the presence of Candidatus *Mycoplasma haemominutum* (15.7%) and *Mycoplasma haemofelis* (9.7%) was observed in 25.4% of the sampled cat population of Seongnam city (4).

The current research has not only shown that the infection rate of *Babesia* spp. among stray cats in Yangju and Gwacheon cities is relatively the high, but it has also confirmed the presence of *Mycoplasma* infection in stray cats in Yangju city. A limitation of this study is that species-level classification of *Rickettia* spp., hemotropic mycoplasmas and *Babesia* spp was not achieved. Therefore, species-level classification should be undertaken along with such genera-based detection in further study.

It is recommended that large-scale studies into the prevalence of infectious agents in all regions of South Korea should be undertaken. In addition, there is a need to integrated such studies with those into the severity of clinical signs and the degree of infection.

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