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< Short Communication>

PCR-based detection of feline vector-borne pathogens in Daejeon

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Abstract

This study was conducted to investigate the detection of various vector-borne pathogens in such cats. A total of 48 stray cats collected in Daejeon were included in this study. The total positive rate of hemotropic mycoplasmas and *Babesia* spp. was 25% and 4%, respectively. It is recommended that species-level classification of hemotropic mycoplasmas and *Babesia* spp. is needed and that a large-scale prevalence study of infectious agents in all the regions of South Korea be conducted.

Key words : Vector-borne disease, Hematopic mycoplasmas, *Babesia* spp., PCR, Stray cat

INTRODUCTION

Many vector-borne pathogens in cats are clinically important, including diseases such as *Anaplasma*, *Babesia*, *Bartonella*, *Elrhichia*, and *Mycoplasma* (Wardrop et al, 1996).

Feline hemotropic mycoplasmas are small epicellular parasites that adhere to the erythrocytes of infected animals and cause feline infectious anemia (Rosenqvist et al, 2016). In clinical fields, hemotropic mycoplasmas are identified by cytological examination or serologic tests; however, these methods have been found to have low sensitivity and specificity, resulting in frequent false negative results (Rosenqvist et al, 2016). Conversely, polymerase chain reaction (PCR) method has a high sensitivity for detection of vector-borne pathogen infection (Alho et al, 2017).

Feline babesiosis is a tick-borne protozoan disease that affects domestic and wild animals and humans worldwide. While the disease has been found in dogs around the world, it is rarely found in cats. Babesiosis is treated with antiprotozoal drugs, while tick control is the best way to prevent infection (Hartmann et al, 2013).

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Epidemiologically, pathogens of such importance in stray cats have never been isolated by PCR in Daejeon, therefore, this study was to conduct to detect the hemotropic mycoplasmas and *Babesia* spp. in stray cats.

MATERIALS AND METHODS

Asymptomatic 48 stray cats (25 females and 23 males; median body weight : 3.3 kg; age : unknown) in Daejeon were included in this study. Blood was collected via the jugular vein and all samples were stored at −80℃ until analysis. Nucleic acid was subsequently extracted from the blood samples and analyzed for the presence of vector-borne pathogens. Total Nucleic Acid Purification Kit (POBGEN^{TM}, KogeneBiotech, Seoul, South Korea) was used to extract both DNA and RNA. Briefly, 140 μL liquid sample was transferred into nuclease-free microcentrifuge tubes, after which 10 μL proteinase K solution (20 mg/mL) and 300 μL Buffer LB1 was added. Sampled were then incubated at 56℃ for 20 min for digestion, after which 250 μL absolute ethanol was added and the samples were vortexed well. Next, approximately 700 μL of the sample was applied to a spin column placed in a 2 mL collection tube

(supplied), after which the samples were centrifuged for 15 s at 8,000 × *g*. The flow-through was subsequently discarded after which and add 600 μL buffer WB1 was added to the spin column, and centrifuged for 15 s at 8,000 × *g* to wash the column. The flow-through was again discarded, after which, 500 μL buffer WB2 was added to the spin column and the samples was centrifuged for 15 s at 8,000 × *g* to wash the column, and flow-through was discarded. After this step was repeated twice, the spin column was placed in a 2 mL collection tube (supplied) and centrifuged at full speed for 1 min. The flow through and the collection tube were then discarded, after which, the column was placed in a new 1.5 mL collection tube and add 50 μL nuclease free was added directly to the spin column membrane. After being allowed to stand for 5 min, the samples were centrifuged for 1 min at 8,000 × g to elute the nucleic acids.

Real time PCR was conducted to detect pathogens using the nucleic acid extracted from the blood as a template. Briefly, the nucleic acid extracted from blood was added into master mix for real time PCR with a 20 μL reaction volume, including pathogen specific primers and a probe, after which, one step RT-PCR was performed by subjecting the samples to 50℃ for 10 min to perform reverse transcription and genetic amplification followed by fluorescent detection by 45 cycles of 95℃ for 10 sec and 60℃ for 30 sec. qPCRs specific for hemotropic mycoplsama and feline babesiosis were designed using 16S rRNA gene sequence data. The assays detected at the level of approximately 10 to 100 copies of a sequence-specific plasmid with target genetic region per reaction. For the determination of the qPCR results, we considered as positive above Ct value 40 after genetic analysis.

Table 1. Number of positive rate of vector-borne pathogens in stray cat in Daejeon

	Hemotropic mycoplasma	*Babesia* spp.
Daejeon		
Number of positive	12	2
Positive rate	25%	4%

RESULTS AND DISCUSSION

We investigated the prevalence of vector-borne pathogens in 48 stray cats found in Daejeon using PCR. All sampled were negative for *Rickettia*, *Batenella, Anaplasma*, *Leptospira*, FeLV, FIV, feline corona virus, and *Erlichia*. However, 12 samples (25%) were positive for hemotropic mycoplasmas and 2 samples (4%) were positive for *Babesia* spp.

Feline hemotropic mycoplasmas are Gram-negative bacteria that cause infected cats to be anemia (Rosenqvist et al, 2016). There are three species, *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum* and *Candidatus Mycoplasma turicensis* (Neimark et al, 2001). *Mycoplasma haemofelis* often induce hemolytic anemia during acute or chronic infection in cats (Cho et al, 2016). Microscopic examination of Wright-Giemsa stained peripheral blood smears is used for diagnosis of hemotropic mycoplasma infection (Crado-Fornelio et al, 2003; Cho et al, 2016). However, this method has low sensitivity; therefore, PCR is now recommended for diagnosis of hemotropic mycoplasmas infection in cats (Cho et al, 2016).

Among 369 domestic cats in southern Brazil, a total of 79 samples (21.4%) were positive (Santos et al, 2014) which is similar to the positive rate observed in the present study. Moreover, the positive rate of hemotropic mycoplasma infection in our study was similar to that observed in Jeonju, South Korea (26%, Cho et al, 2016).

Outdoor access is a well-known risk factor for hemotropic mycoplasma infection, and some prevalence studies in the United States and Ireland showed that feral cats had a higher prevalence rate of hemoplasmas than client-owned cats (Jensen et al, 2001; Juvet et al, 2010).

Feline babesiosis is a tick-borne protozoan disease commonly found in dogs around the world, but rarely found in cats. In our study, the positive rate by PCR was 4% in stray cats. In a study of stray cats in Bangkok, Thailand, the positive rate by PCR was only 1.4% (Simking et al, 2010). These two studies suggest that there is a low low prevalence of *Babesia* infection in stray cats. Babesia spp. which have a low prevalence in feral cats, should be considered in anemic patients

with doubtful history, and confirmed by PCR. Moreover, follow-up studies of the epidemiology or the route of infection of these diseases are needed, especially those investigating the relationship between groups of client-owned cats and stray cats because the pathogens are so prevalent in the latter group. It should be noted that this study was limited in that species-level classification of hematopic mycoplasma and *Babesia* spp. was not been performed. Therefore, species-level classification should be conducted in future studies.

CONCLUSION

In this study, we investigated the prevalence of various vector-borne pathogens. Of these infectious agents, the positive rate of hemotropic mycoplasmas was high, but the positive rate of *Babesia* spp. was relatively low. It is recommended that a large-scale prevalence study of infectious agents in all the regions of South Korea be conducted.

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