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Prevalence of *Babesia* spp. in dogs of Seogwipo-si, Jeju-do, South Korea

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Abstract

Dogs with canine babesiosis may present with wide variation in the severity of clinical signs, ranging from a hyperacute, shock-associated, hemolytic crisis to an inapparent, subclinical infection. Dogs typically present with the acute form of babesiosis, which is characterized by general findings such as pyrexia, weakness, mucous membrane pallor, depression, hemorrhagic anemia. This study was conducted to investigate the prevalence of *babesia* spp. infection in dogs of Seogwipo-si. A survey of canine *babesia* spp. infections among 173 dogs in Seogwipo-si was performed from July 2008 to August 2008. Blood samples were collected from dogs raised outdoors through cephalic or jugular vein and Babesia spp. was diagnosed by examination of blood smear stained with Giemsa stain. Of 173 dogs, 9 dogs (5.2%) were infected with the *babesia* spp. This result was a little lower than the prevalence of *Babesia* spp. in dogs of other areas.

Key words : Prevalence, *Babesia* spp., Dog, Giemsa stain, Seogwipo-si

INTRODUCTION

Canine babesiosis is a tick-borne disease caused by protozoal parasites, *Babesia giboni* and *B. canis*. They infect the red blood cells of dogs and typically cause hemolytic anemia (Miyama et al, 2005). *Babesia* spp. (*B.* spp) is distributed in Asia, Africa, Europe, the Middle East, and North America (Conrad et al, 1991).

The incubation period is 3 to 8 days. The body temperature goes up to about 39～40°C when acutely infected and intermittent fever is a common symptom in long-term infection. In addition, the hemoglobin content and the hematocrit value can be also dropped by the diminution of the erythrocytes.

However, only a few epidemiological and clinical studies have been reported on canine babesiosis. In Korea. According to the reports, *Babesia* spp. has been detected in Kyungju (Son, 1964), Kyungbook (Son, 1964), Pusan (Son, 1964), Jeonbook (Chae et al, 1989), Jinju (Shin and Kim, 2000) and Jeju (Han and Kim, 1982).

Treatment or spontaneous recovery from an acute infection frequently fails to clear the organism from the host, resulting in a carrier stage. The recovered animals have chance to become the reservoir for tick-transmitted infections and they are at risk for recrudescent infection (Farwell GE et al, 1982). In previous studies, the infection rate of *Babesia* spp. was a little higher than in other tick-restricted areas. For examples, among eastern countries, the prevalence of *Babesia* spp. infection was 30.4% in Japan in 2004, and in Gangwon and Gyunggi of South Korean provinces, 1.8% of dogs were positive by PCR and ELISA (Miyama et al, 2005; Song, 2004). 18.8% of dogs in Brazil were also found positive by the indirect fluorescent antibody test (IFAT) (Maia et al, 2007). On the flip side, 2.7% were positive in Jeju in 1972 by Giemsa stain (Han and Kim, 1982).

The object of this study was to identify the incidence

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of *Babesia* spp. infection in dogs raised around Seogwi-po-si, and the blood samples of dogs were investigated from July, 2008 to August, 2008.

MATERIALS AND METHODS

The study was conducted for a period of 2 months from July to August in 2008 in the College of Veterinary Medicine, Cheju National University.

Experimental animals

A total of 173 dogs which were raised outdoors in Seogwipo-si were selected randomly for blood collection.

Blood samples collection

About 1～3ml of blood was collected from cephalic or jugular vein of each dog with the help of sterile disposable syringe and needle. As soon as blood samples were collected, the whole blood samples were transferred to the anticoagulant tube containing EDTA (0.02g/10ml of blood) and they were examined at the parasite laboratory.

Table 1. The number of dogs infected in *Babesia* spp. in each region in Seogwipo-si

Area	Numbers examined	Numbers infected	Infection rate (%)
Wi-mi	7	0	0
Sin-heung	13	1	7.7
Dae-jung	8	1	12.5
Pyo-seon	12	0	0
Sin-san	6	0	0
Sung-san	18	1	5.6
An-deok	10	1	10
Sek-dal	7	0	0
Hyo-don	12	0	0
Ha-rye	7	0	0
Sang-hyo	11	1	9.1
Ho-guen	11	0	0
To-pyung	16	2	12.5
Sang-ye	6	0	0
Hoi-su	4	1	25
Wol-pyung	6	0	0
Sin-hyo	12	1	8.3
Ha-hyo	7	0	0
Total	173	9	5.2

Diagnostic methods

Giemsa staining was used for diagnosis of *Babesia* spp. After slides were prepared, the thin peripheral blood smear was fixed with a commercial methanol. And then reagents, Giemsa stain and Giemsa buffer (diluted with distilled water 1:19) were supplied on the thin blood smear slide. Giemsa stain was applied to the slides for about 30～40 minutes. After staining, the slide was washed out by flowing in tap water. Finally, the slide was observed under a light microscope ($\times$1,000) to determine the presence of the parasite in erythrocytes.

RESULTS

Prevalence of *Babesia* spp. in dogs of Seogwi-po

As shown in Table 1, the overall prevalence of *Babesia* spp. in dogs was 5.2% (9 dogs) among 173 dogs by Giemsa staining. In the concrete, 18 areas of Seogwipo-si were investigated for this study. The number of dogs infected with *Babesia* spp. in each region in Seogwipo-si has also been shown in Table 1. One of 13 dogs (7.7%) were positive in Sin-heung area, 1 of 8 dogs (12.5%) were positive in Dae-jung area and 1 of 18 dogs (5.6%) were positive in Sung-san area. And Babesia spp. were observed by Giemsa stain in 1 of 10 dogs (10.0%) in An-deok, 1 of 11 dogs (9.1%) in Sang-hyo, 1 of 4 dogs (25.0%) in Hoi-su, 1 of 12 dogs (8.3%) in Sin-hyo, respectively. Two cases of 16 dogs (12.5%) were positive in To-pyung area.

This showed that dogs infected with *Babesia* spp. were distributed in the every part of Seogwipo-si, even if the number of the infected was not big.

Sexual distribution

Sexual distribution of canine babesiosis in Seogwipo-si presented in the Table 2 revealed that 5 of 92 dogs

Table 2. The number of dogs infected with *Babesia* spp. by sex

Sex	No. of Positive	Total
Male	5	92
Female	4	81
Total	9	173

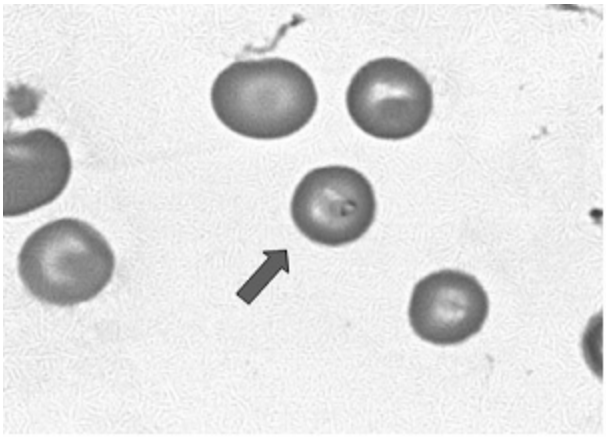

Fig. 1. Photomicrography of *Babesia* spp. stained with Giemsa stain from thin blood smear (arrow).

(5.4%) were positive in the case of male, while 4 of 81 dogs (4.9%) were positive in the case of female, indicating that there was no significant difference between male and female.

Morphologic characteristic of *Babesia* spp.

The micrography of *Babesia* spp. was confirmed by the examination of Giemsa stained peripheral blood smears. Following Fig. 1, the parasites were pleomorphic (vary in shape and size) and could be vacuolated, and did not produce pigment.

DISCUSSION

In the previous studies, the prevalence of *Babesisa* spp. was a little higher than in other tick-restricted areas. For examples, in case of eastern counties, 30.4% among all the dogs were positive in Japan in 2004 (Miyama et al, 2005). And in Gangwon and Gyunggi of Korea provinces, 1.8% of dogs were positive by PCR and ELISA (Song, 2004). In addition, 18.8% of dogs in Brazil were also found positive by the indirect fluorescent antibody test (IFAT)(Maia et al, 2007). On the flip side, 11 among 395 dogs (2.7%) were positive in Jeju 1972 by Giemsa stain (Han and Kim, 1982). In this study, 9 among 173 dogs (5.2%) were babesia-positive by Giemsa staining.

This study shows that dogs infected *Babesia* spp. are distributed in the every part of Seogwipo-si, even if the number of the infected is not that big. The way to definitively diagnose the disease in this report was the visualization of the parasite in erythrocytes by using a light microscope.

However, It is hard to detect *Babesia* in the dogs by microscopy because when the dogs have been infected with *Babesia* spp., there are lots of chances to have immunity for *Babesia* spp., and dogs which survive from an acute crisis develop latent infection (Farwell et al, 1982). Latent infection is a delicate balance between the host immune response and the parasite's ability to induce clinical disease (Conrad et al, 1991).

And *Babesia* spp. might not be detected by blood smear because it is not an ideal method. As discussed previously, diagnosis of babesiosis in dogs by blood smear seemed to be difficult, and this study showed how hard it is to determine a reasonable infection rate of *Babesia* spp.

For these reasons, it seemed that infection rate in these blood smear samples was lower than the previous studies.

Considering the facts, it is thought that we need more advanced methods for the accurate diagnosis of *Babesia* spp. And it is thought that a number of ticks as carriers were parasitic on dogs around Seogwipo-si because Seogwipo-si was somewhat countryside and the subjects of this study were dogs raised outdoors so they had a high rate of exposure to ticks. Thus, surveys of ticks as spontaneous vectors of *Babesia* spp. will be needed to evaluate the actual distribution of *Babesia* spp. in Seogwipo-si. In addition, we need to make sure if the activity of ticks can be changed following the temperature in each season and the rate of infection rate in outdoor dogs was affected.

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