pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 34(3) : 185-188 (2017)
http://dx.doi.org/10.17555/jvc.2017.06.34.3.185

Journal of Veterinary Clinics
www.ksvc.or.kr

Seroprevalence of *Babesia gibsoni* in Companion Dogs in Korea by Enzyme Linked Immunosorbent Assay using Recombinant BgTRAP Antigen

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(Received: January 04, 2017 / Accepted: June 03, 2017)

Abstract : This study assessed the seroprevalence of *Babesia gibsoni* in companion dogs in Korea by enzyme linked immunosorbent assay using recombinant BgTRAP antigen. Dogs were randomly selected from those admitted for various reasons to local private veterinary hospitals and the Animal Medical Center of Chonbuk National University. With the owners' permission, extra blood was drawn from each dog for serological assays. Of the 188 selected dogs, seven (3.72%) were positive for *B. gibsoni*, including six of 167 (3.59%) indoor and one of 12 (8.33%) outdoor dogs. Of the seven dogs positive for *B. gibsoni*, four were aged > 10 years, two were < 1 year, and one was between 1 and 10 years; and two were Yorkshires and one each was Shih-tzu, Maltese, Pekinese, beagle and mixed. Concurrent diseases or chief complaints were anemia in two dogs, both of which had a history of confirmed babesiosis by polymerase chain reaction, and non-anemic diseases in five. Geographically, four dogs were from Jeonbuk/Jeonju, and one each from Seoul, Gyounggi-do, and Jeonnam/Gwangju. To our knowledge, this is the first report of companion dogs in Korea being seropositive for *B. gibsoni*. Serologic screening of subclinical or carrier dogs can detect this potentially dangerous disease and assess its epidemiology.

Key words **:** *Babesia gibsoni*, ELISA, BgTRAP, dog.

Introduction

Canine babesiosis is an important worldwide tick-borne disease caused by blood parasites belonging to the genus *Babesia*. Species causing canine babesia include *B. canis* and *B. gibsoni.* Subspecies of *B. canis* include *B. canis canis, B. canis vogeli,* and *B. canis rossi*, which are transmitted by *Rhipicephalus sanguineus, Dermacentor spp., Haemaphysalis leachi,* and *Hyalomma plumbeum* (9). *B. canis* has been reported throughout the world, including in Africa, Asia, Australia, Europe, and the Americas. *B. gibsoni* can be transmitted by *Haemaphysalis bispinosa, H. longicornis,* and *R. sanguineus* (16). Infections can also occur via blood transfusion, by contact with needles used for vaccination, and by contact with the surgical instruments used for ear trimming and tail docking surgery (13). Injuries during dog fights have been reported to be a major route of infection of American pit bull terriers (13). *B. gibsoni* infection was first reported in 1910 in India, and mostly occurs in India, Southeast Asia, and Egypt (2,4).

Diagnostic methods include blood smear examination, serological tests, and polymerase chain reaction (PCR) (9). Examination of blood smears by light microscopy is an easy and simple method, but the organisms can only be detected during the acute phase of infection or at high parasitemia levels. In one study, nine of 173 outdoor dogs were found to be positive by this method (15). PCR is the most sensitive and specific means of detection with semi-nested PCR differentiating between *B. canis* and *B. gibsoni* (1). In Korea, this method has been utilized to assess the prevalence of babesiosis in dogs (11,18).

Canine babesiosis can also be diagnosed serologically, using enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA), a sensitive method of detecting chronic and occult infections, as well as low level parasitemia. Generally, IFA is more commonly used for canine babesiosis, with PCR identification required to differentiate between *B. canis* and *B. gibsoni*, due to the cross reactivity of relevant antibodies used in IFA (9). One study reported that 5.8% of American Staffordshire terriers and 14.7% of American pit bull terriers were positive by both IFA and ELISA (9). In Korea, 7.8% of 774 mixed dogs, 81.3% of 96 fighting dogs, and 15.6% of 96 German shepherd dogs were positive by both IFA and ELISA (9,17).

Among the recombinant babesia antigens used in ELISA are rP50, rP29, GST-P50t, rBgSA1, rBg12D, BgRAP-1, rBg32, rBgP57, rBgTRAP (6), rBg12 (7), and BgRAP-1s (20). Of these assays, ELISA using BgTRAP has been reported the most sensitive (6). An assay of dogs in east Japan in 2005 using the recombinant GST-P50t antigen ELISA method reported that 30.4% of these dogs were positive (14).

Although one report described utilizing both IFA and ELISA to assay dogs in Korea, that report did not describe the antigen used in the assay (17). Therefore, to our knowledge, no serological study using BgTRAP ELISA has been performed to date in Korea. This study was performed to

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serologically assess the prevalence of canine babesiosis in companion dogs in Seoul and Jeollabukdo in Korea using recombinant BgTRAP ELISA. Results of this study should provide an epidemiological and clinical basis for monitoring and/or treating this disease.

Materials and Methods

Samples

The study included 188 dogs randomly selected from those that presented to local veterinary hospitals and the Animal Medical Hospital of Chonbuk National University from May to December 2014. Characteristics recorded included breed, age, sex, and lifestyle (indoors or outdoors). After obtaining permission from owners to draw extra blood for serologic assays, 5 ml blood was drawn from the jugular vein of each animal into plain tubes or serum separating tubes (SST). The tubes were centrifuged at 1500 × g for 5 min, and the serum kept frozen until assayed.

ELISA

The antigen used in the ELISA was thrombospondin-related adhesive protein of *Babesia gibsoni* (BgTRAP), which was kindly contributed by Professor Fukumoto Shinya of the National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan. The ELISA with rBgTRAP was essentially performed as described previously (5). Each well of a 96-well microplate (Nunc, Denmark) was coated with rBgTRAP (50 ng/well) and GST (50 ng/well), both in an antigen coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) for one hour at room temperature. The plates were blocked with 3% (w/v) skim milk for 30 min at room temperature (20°C). After washing, the plates were incubated with serum samples, diluted 1:400. The plates were again washed and incubated with horseradish peroxidase conjugated sheep anti-dog IgG heavy chain antibody (BETHYL, Laboratories, Inc; 1:50000) and TMB substrate (BETHYL). The color was allowed to develop at room temperature, and the optical density (OD) was measured using the Epoch microplate reader (Bio Tek, Vermont, USA) at 450 nm, and analyzed by Gen5 (Bio Tek, Vermont, USA).

The ELISA titer was expressed as the reciprocal of the maximum dilution that showed an ELISA value equal to or greater than 0.046, the difference in absorbance between that for the antigen (GST-BgTRAP)-containing well and that for the control antigen (GST)-containing well. The absorbance of 0.046 was calculated by determining the mean ± three standard deviations (SDs) of four specific pathogen-free (SPF) dog sera.

Results

Serum samples were obtained from 188 dogs, including 31 Shih-tzus, 41 Maltese, 16 poodles, 17 Yorkshire terriers, 24 mixed breeds, nine Pomeranians, nine Pekinese, seven cocker spaniels, seven miniature schnauzers, four miniature pinschers, three dachshunds, four chihuahuas, two spitzes, three beagles, one boxer, five golden retrievers, one Jindo, one Alaskan malamute, one Poong-san, one pointer, and one of unknown breed (Table 1). Geographically 66 samples were from Seoul, 18 from Kyoung gi and Incheon, seven from Chungnam and DaeJeon, 86 from Chonbuk and Jeonju, nine from Chonnam and Gwangju, and two from unknown areas. Of the 188 dogs, 167 were classified as indoor, and 12 as outdoor animals, with nine unknown.

Table 1. Babesiosis exposure by demographic characteristics in dogs based on detection of pathogen-specific antibodies

Factors	Number of samples	Number of positive samples	Seroprevalence (%)
All samples	188	7	3.73
Lifestyle			
Outdoor	167	6	3.59
Indoor	12	1	8.33
Unknown	9		
Age (years)			
< 1	18	2	11
1-10	76	1	1.3
10 <	88	4	4.5
Sex			
Male	91	2	2.1
Female	95	5	5.2

Of the 188 specimens, seven (3.73%) were positive on ELISA, including six of the 167 (3.59%) indoor dogs and one of 12 (8.33%) outdoor dogs. The remaining nine unclassified dogs were negative.

The seven positive dogs included four of the 88 (4.5%) dogs aged > 10 years, two of the 18 (11%) dogs aged < 1 year, and one of the 76 (1.3%) dogs aged between 1 and 10 years. Two of the positive dogs were Yorkshire terriers, with one each being Shih-tzu, Maltese, Pekinese, beagle, and mixed. Chief complaints at first admission to hospital were anemia and renal failure in two dogs each and heart failure, mastitis, and mammary tumor in one each. Both dogs with anemia were also positive for *B. gibsoni* on PCR analysis. None of the other five positive dogs had a history of exposure to ticks or transfusion.

Geographically, four of the 86 dogs (4.65%) from Chonbuk and Jeonju were positive, along with one of 66 (1.52%) from Seoul, one of 18 (5.56%) from Kyounggi and Incheon, and one of 9 (11.1%) from Chonnam and Gwangju.

Discussion

This study demonstrated serologically that seven of 188 companion dogs in Korea were positive for *B. gibsoni* by ELISA using BgTRAP. To our knowledge, this is the first study on *B. gibsoni* infection in companion dogs in Korea, and the first serological survey using BgTRAP ELISA in Korea. In areas where tick vectors are not endemic, *B. gibsoni* is transmitted between dogs mainly by biting during dogfights; less frequently, *B. gibsoni* is transmitted transplacentally, or by transfusion and through surgical apparatus (13).

Ticks such as *Haemaphysalis bispinosa* and *H. longicornis* have been detected in dogs with canine babesiosis in Japan, as well as in other Asian countries including Korea. The prevalence of *B. gibsoni* in Korea has been reported to be 1.8-13.6% by PCR and 5.1% by microscopic examination (15,18). In the only previous study using IFA and ELISA in Korea, 60 of 774 mixed dogs (7.8%), 78 of 96 fighting dogs (81.3%) and 15 of 96 military dogs (15.6%) were positive for B. gibsoni by IFA. By ELISA, however, 24% of mixed dogs, 83.3% of fighting dogs, and 38.5% of military dogs were positive (17).

The significantly lower positivity rate in this study (3.73%) was likely due to the dogs selected, as they consisted of different breeds and lifestyles; moreover the inherent sensitivity of ELISA may have contributed in part to the lower positivity rate (1,9). Outdoor military dogs, fighting dogs, and mixed breed dogs show higher positivity rates, as they are at greater risk of exposure to ticks and bite transmission (9). Bite transmission has been reported as a major route for the transmission of *B. gibsoni* in fighting dogs. In this study most of the dogs were indoor companion dogs, and had lower opportunity to tick exposure, which may explain the lower rate of positivity in this study (11).

The effects of the antigens used in these studies cannot be determined, because information on the antigen used in the previous study is unavailable. A recent study in China on the seroprevalence of *B. gibsoni*, using the same antibody in pet dogs, showed results consistent with ours (2). Although recombinant BgTRAP ELISA is less sensitive than PCR, this method can detect antibody during the period in which antibody titers persists, whereas PCR can only detect DNA during parasitemia (6). Positive antibody titer indicates exposure to *B. gibsoni*, which can be interpreted as prior infection, subclinical infection or chronic carrier state. Antibody to *B. gibsoni* was found to develop eight days after infection, persisting for 2465 days (6).

Affected dogs become chronic carriers, with antibody titers persisting for a longer period of time because parasitemia can persist up to 38 months (4,8). Chronically infected dogs can be infected subclinically; develop intermittent fever, lethargy, and weight loss; be poorly responsive to treatment and have recurrent infection. Immunosuppression can precipitate infection after months or years, and disease can worsen into glomerulonephritis or polyarthritis (3,12,21).

The ELISA screening method described in this report may be useful for detecting and managing high risk subjects. This is especially important in dogs that develop immune mediated hemolytic anemia, and receive immunosuppressive treatment, such as steroids or splenectomy, because babesiosis can recur in these immunosuppressed animals (10). ELISA can also be utilized in managing subclinical carrier dogs because *B. gibsoni* can be transmitted via blood transfusion. One report described a two-year-old German shepherd that developed babesiosis after receiving blood from a healthy American pit bull terrier (19).

BgTRAP ELISA is a sensitive and specific test. Of dogs positive by PCR, 79.5%, 59.8%, 66.4%, and 60.7% were positive by ELISA using rBgTRAP, rBgP50, rBgSA1, and rBgP32 antigens, respectively (6). Of dogs negative by PCR, 10.3%, 9.35%, 10.3% and 11.2%, respectively, were ELISA positive using these same antigens. Recombinant BgTRAP antigen did not cross react in dogs experimentally infected with *B. canis canis, B. canis vogeli, B. canis rossi,* and *N. caninum* (6).

In this study, PCR was performed in all dogs. Two dogs were anemic at first admission to hospital, with both being p ositive by PCR and microscopic examination. Blood samples for ELISA were drawn from these dogs approximately one month and three months, respectively, after initial diagnosis. Of the other five dogs positive by ELISA, none had a prior history of canine babesiosis.

In conclusion, this study showed that BgTRAP ELISA could detect *B. gibsoni* in both anemic and non-anemic companion dogs in Korea. This method may be useful in screening subclinical or carrier dogs in clinics and for epidemiological studies.

Acknowledgements

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No: PJ01198902)" Rural Development Administration, Republic of Korea. The authors thank Professor Xuenan Xuan for kindly donating the recombinant BgTRAP antigen for this study.

Conflict of Interest

We have no conflict of interest related to this work.

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