

Serological survey of *Borrelia* infection of dogs in Sapporo, Japan, where *Borrelia garinii* infection was previously detected

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ABSTRACT. A serological survey of *Borrelia* infection of dogs was performed in Sapporo, Japan, where *Borrelia garinii* infection in dogs was detected in 2011. A total of 314 serum samples were collected from dogs that visited three animal hospitals in Sapporo from 2012 to 2014. The two-step evaluation method, involving screening ELISA followed by Western blot analysis, was used to detect antibodies against *Borrelia* species. A total of 34 samples were positive by ELISA. Among those 34 samples, 32 were positive for *Borrelia* spp. by Western blot. These findings suggest that the 32 dogs (10.2%) generated antibodies against *Borrelia burgdorferi* sensu lato, such as *B. garinii* or *B. afzelii*. Antibody positivity was 7.6% and 13.3% for dogs living in urban and rural areas, respectively. Dogs with a history of tick infestation showed a positive rate of 16.7%, which was higher, although not significantly, than the 6.7% among dogs without a history.

KEY WORDS: *Borrelia burgdorferi* sensu lato, canine, Sapporo, serosurvey

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Lyme disease is a zoonotic infectious disease caused by *Borrelia burgdorferi* sensu lato. *Borrelia burgdorferi* sensu stricto is the most pathogenic bacteria among those which cause Lyme disease in both human and dogs, and is distributed mainly in North American and European countries [1, 4, 5, 8, 19, 20]. While Lyme disease has also been reported in Japan, *Borrelia garinii* and *Borrelia afzelii* are thought to be the main causative agents [12, 13, 23]. Canine Lyme disease was also reported in dogs in Hokkaido, the northern island of Japan, in the 1990s [2, 3]. At that time, *B. burgdorferi* was considered the pathogen. In 2011, two dogs which presented with sudden astasia, anorexia and fever more than 40°C were diagnosed with Lyme disease related to *B. garinii* infection in Sapporo, Hokkaido [9]. Because diagnostic laboratories for Lyme diseases caused by *B. garinii* and *B. afzelii* are limited in the veterinary setting in Japan, little is known about the epidemiology of canine Lyme disease. Gaining further insight into *Borrelia* infections of dogs in Sapporo, Japan, where canine Lyme disease caused by *B. garinii* was previously reported, is a pressing issue. To this end, the present study aimed to conduct a serological survey of *Borrelia* infection of dogs in Sapporo, Japan.

In total, 314 serum samples were collected from dogs that visited three animal hospitals in Sapporo, Japan, from 2012 to 2014. Most dogs visited the hospitals for health checkups, vaccinations, filarial control, tick control, castration and dental scaling, and were considered clinically healthy. Only four

dogs presented with fever, anorexia or ataxia, clinical signs related to *Borrelia* infection. Living environment (urban or rural), sex and breed were recorded for each dog, and information on the history of tick infestation was obtained via questionnaire from dog owners. Serum samples were kept at –20°C until use.

The 2-step evaluation method was used to detect antibodies against bacteria belonging to *Borrelia burgdorferi* sensu lato as recommended by the Centers for Disease Control and Prevention [6]. First, samples were screened by enzyme immunoassay. The *Borrelia* Dog IgG-ELISA Kit[®] (Mikrogen, Neuried, Germany) was used to detect IgG antibodies against pathogenic *Borrelia* species, including *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Samples which were positive or unclear were selected for evaluation using the second step, which involved use of the recomBlot *Borrelia* canis IgG[®] kit (Mikrogen). Scoring was based on the presence of antibodies against each antigen, with a score of ≥ 7 defined as positive.

Fisher's Exact test was used to analyze the relationship between questionnaire items and antibody positivity. The critical probability was set at $P=0.05$.

We examined 314 canine serum samples for IgG antibodies against pathogenic *Borrelia* species using the two-step evaluation method; 34 (10.8%) were positive by screening ELISA. Among these, 32 were further positive (≥ 7 points) for pathogenic *Borrelia* spp. by Western blot analysis. Since IgG antibodies against *Borrelia* can remain in the dogs for more than a year after exposure to the pathogen *Borrelia* [11], they are reliable markers for exposure. These findings suggested that the 32 positive samples (10.2%) among 314 might include antibodies against pathogenic *Borrelia* species, including *B. burgdorferi* sensu stricto, *B. garinii* or *B. afzelii*. As *B. burgdorferi* sensu stricto has never been detected in Japan, *B. garinii* or *B. afzelii* is more possible species of exposure. Both *B. garinii* and *B. afzelii* are known to

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Table 1. Proportion of dogs exposed to each determinant of positive antibodies against *Borrelia* species as determined by the recom-Blot *Borrelia* test

	Number of dogs	Number of positive dogs	Percent positivity	<i>P</i> value*
Living environment				
Urban	171	13	7.6	0.1331
Rural	143	19	13.3	
History of tick infestation				
Yes	48	8	16.7	0.0717
No	163	11	6.7	
Unknown	103	13	12.6	
Sex				
Males (including castrated males)	163	16	9.8	0.9227
Females (including spayed females)	145	16	11.0	
Unknown	6	0	0.0	
Breed				
Mix	41	7	17.1	0.6824
Shih Tzu	27	4	14.8	
Miniature Dachshund	34	4	11.8	
Labrador Retriever	17	2	11.8	
Toy Poodle	28	3	10.7	
Chihuahua	10	1	10.0	
Yorkshire Terrier	10	1	10.0	
Shiba	23	2	8.7	
Others**	134	8	6.0	

*Fisher's Exact test, **Others include breeds for which fewer than 10 animals were assessed.

be pathogenic *Borrelia* species in Japan and are distributed mainly in northern Japan [12]. The vector of both *B. garinii* and *B. afzelii* is thought to be *Ixodes persulcatus* [14, 15, 17], which is distributed mainly in the Tohoku and Hokkaido areas in northern Japan [10, 21, 22]. This tick species is also the most dominant tick species of dogs in Hokkaido [10]. Recently, human clinical case of *Borrelia miyamotoi* has been reported in Japan [16], which was originally detected from *I. persulcatus* ticks [7]. Because the cross reactivity of antibodies against *B. miyamotoi* with the antigens used in the present study is unknown, it is also possible that the 32 positive dogs might be exposed against the *B. miyamotoi*.

Most dogs (310 of 314, 98.7%) examined in the present study showed no clinical symptoms and were considered clinically healthy. Only four dogs presented with anorexia or fever, which could be considered symptoms of Lyme disease. One dog among the four was positive for antibodies against pathogenic *Borrelia* by the two-step evaluation method. This dog was diagnosed with pancreatitis by clinical and laboratory tests. As peripheral blood from the dog was not examined for *Borrelia* infection by PCR in this study, the relationship between *Borrelia* infection and its clinical symptoms could not be determined. In earlier and acute stage of the Lyme disease, circulating IgM is usually more superiorly detected than IgG. Because IgG against *Borrelia* spp. was targeted in this study, the positive results don't correlate with the present illness.

The relationship between positivity for *Borrelia* antibodies and factors that appear relevant to tick-borne *Borrelia* infection was analyzed (Table 1). Antibody positivity in dogs living in urban and rural areas was 7.6 and 13.3%, respec-

tively, with no significant difference. Positivity in dogs with a history of tick infestation was 16.7%, which was higher than the 6.7% observed in dogs without a history. Although this difference was not significant, it should be noted that 6.7% of dogs without a history of tick infestation were exposed to the *Borrelia* pathogen. This finding suggests that dog owners might not be aware that their dogs carry ticks. Clinical veterinarians should note that even if owners do not indicate tick infestation, dogs may have been exposed to pathogenic *Borrelia*.

Positivity for *Borrelia* antibodies did not significantly differ between males (9.8%) and females (11.0%). This may reflect the fact that most of the examined dogs were not free roaming and thus there were fewer differences in behavior between sexes. While some breeds have a higher positivity for antibodies against *Borrelia* (Mix, 17.1%; Shih Tzu, 14.8%; Miniature Dachshund, 11.8%; and Labrador retriever, 11.8%), there was no significant difference between breeds.

Borrelia is a zoonotic pathogen of both humans and dogs, and dogs are known to be potential sentinel animals for *Borrelia* infection [18]. The results of the present study suggest that companion dogs living in the Sapporo area are at risk of infection by *Borrelia burgdorferi* sensu lato, such as *B. garinii* and *B. afzelii*, and that owners who walk with their dogs are also at risk of *Borrelia* infection.

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