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Serological evidence of *Anaplasma* spp., *Borrelia burgdorferi* and *Ehrlichia canis* in dogs from the Republic of Korea by rapid diagnostic test kits

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ABSTRACT

Background: Emergent and re-emergent canine tick-borne infections are attracting increasing attention worldwide. The rise in pet ownership and the close relationship between dogs and their owners are the most concerning factors because dogs may act as competent reservoirs for human tick-transmitted infectious agents.
Objectives: This study contributes to the epidemiological surveillance of canine tick-transmitted infections with zoonotic risk in the Republic of Korea (ROK) by investigating the seroprevalence of the pathogens, *Anaplasma* spp., *Borrelia burgdorferi*, and *Ehrlichia canis*.
Methods: Four hundred and thirty whole blood samples from domestic dogs were collected in seven metropolitan cities and nine provinces in the ROK and tested using SensPERT Ab test kits (VetAll Laboratories®) to detect seroreactive animals.
Results: The seroprevalence rates identified were 9.8% (42/430) for *Anaplasma* spp., 2.8% (12/430) for *B. burgdorferi*, and 1.4% (6/430) for *E. canis*. The risk factors evaluated in this study that could be associated with the development of a humoral immune response, such as sex, age, and history of tick exposure, were similar. There was only one exception for dogs seroreactive to *Anaplasma* spp., where the risk factor "tick exposure" was statistically significant ($p = 0.047$).
Conclusions: This serological survey exhibited the widespread presence of *Anaplasma* spp., *B. burgdorferi*, and *E. canis* throughout the ROK. Hence, dogs may play a key role as the sentinel animals of multiple zoonotic infectious agents in the country.

Keywords: *Anaplasma*; *Borrelia burgdorferi*; *Ehrlichia canis*; seroprevalence; dog

INTRODUCTION

The incidence and geographical distribution of canine vector-borne diseases (CVBDs) have increased globally [1,2]. Ticks are the main vector of pathogen transmission to animals and have been associated with the dissemination of numerous bacterial, parasitic, and viral diseases [3]. Among the bacterial pathogens that these hematophagous arthropods can transmit to dogs, the Anaplasmataceae, Spirochaetaceae, and Rickettsiaceae families are of primary medical and veterinary concern [4].

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Conflict of Interest
All authors have no potential conflicts of interest.

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One of the widely distributed species of the Anaplasmataceae family is *A. phagocytophilum*, a bacterium that primarily infects neutrophils, even though it can occasionally be found in eosinophilic granulocytes [5], and is transmitted by *Ixodes* ticks, which are also competent vectors for *Borrelia burgdorferi* [6]. *A. phagocytophilum* is the causative agent of canine granulocytic anaplasmosis (CGA), a disease with clinical manifestations ranging from self-limiting to severe [7,8]. On the other hand, *A. platys*, the bacterium responsible for causing infectious canine cyclic thrombocytopenia (ICCT) [9], infects platelets and is assumed to be transmitted by *Rhipicephalus sanguineus* ticks, a vector of multiple pathogens, including *Ehrlichia canis* [10]. In North America, *A. platys* is considered less pathogenic than *A. phagocytophilum* [11].

Interestingly, among the Anaplasmataceae family, *E. canis* was the first agent of monocytic ehrlichiosis to be identified in dogs [4]. Experimentally, an infection with *E. canis* results in acute, subclinical, and chronic disease states, with dogs exhibiting various clinical signs and laboratory abnormalities during the stages of infection [11,12].

Regarding infections with the spirochete, *B. burgdorferi*, the causative agent of Lyme borreliosis, is less frequent in dogs than in humans, causing milder clinical signs in dogs. Only approximately 5% to 10% of dogs exposed to infected ticks develop clinical borreliosis [13,14].

Because *Anaplasma* spp., *E. canis*, and *B. burgdorferi* bacteria can infect dogs and humans, this research makes a key contribution to public health surveillance of tick-borne diseases in the ROK by investigating their seroprevalence rates and identifying the geographical distribution of seroreactive animals.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted in seven metropolitan cities and nine provinces in the ROK (**Table 1**). Four hundred and thirty whole blood samples were collected at different veterinary hospitals and clinics and then referred to the Laboratory of Veterinary Internal Medicine of Seoul National University, ROK, between April 2019 and December 2020. All animals tested in

Table 1. Geographic location of the veterinary hospitals and clinics where whole blood samples were collected from dog patients between 2019 and 2020 in the Republic of Korea

Location	Name	No. of collected samples
Metropolitan cities (n = 7)	Seoul	146
	Incheon	8
	Daejeon	7
	Daegu	32
	Ulsan	9
	Busan	15
	Gwangju	1
Provinces (n = 9)	Gyeonggi-do	140
	Gangwon-do	7
	Chungcheongbuk-do	10
	Chungcheongnam-do	10
	Gyeongsangbuk-do	2
	Gyeongsangnam-do	15
	Jeollabuk-do	19
	Jeollanam-do	8
	Jeju-do	1
Total		430

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this study were dogs with a history of tick bites or clinical signs suggesting a presumptive diagnosis of tick-borne disease. The whole blood samples were collected in capillary blood collection tubes containing EDTA anticoagulant and transported under a cold chain to the laboratory for further processing.

Serologic analysis

Serological testing was conducted using SensPERT Ab test kits to detect the antibodies against *Anaplasma* spp. (*A. phagocytophilum*/*A. platys*) (sensitivity and specificity of 100% vs. IFA [Indirect Immunofluorescence assay]), *B. burgdorferi* (sensitivity and specificity of 100% vs. IFA), and *E. canis* (sensitivity of 97.7% and specificity of 100% vs. IFA). The manufacturing company (VetAll Laboratories®, Goyang-si, ROK) provided these rapid immunochromatographic test kits for research purposes.

The serological test procedure consisted of adding one drop (10 μL) of whole blood to the specimen well using a dropper as a pipette. Until the whole blood drop was absorbed completely, two drops (80 μL) of buffer were dispensed on it. The results were interpreted 10 min later according to the manufacturer's instructions.

Statistical analysis

A Chi-squared test was used to analyze the different risk factors that may be associated with the humoral immune response. This test was performed using the GraphPad Prism software package (v. 5.04; GraphPad Software, Inc., USA). The results were considered statistically significant when the p value was ≤ 0.05.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Seoul National University Animal Care and Use Committee (No. SNU-190524-2-1). Informed consent was obtained from the owners of all animals involved in the study.

RESULTS

From the three tick-borne pathogens under study, many dogs were seroreactive to antibodies against *Anaplasma* spp. (*A. phagocytophilum*/*A. platys*). The overall seroprevalence rate identified for this bacterium was 9.8% (42/430). Based on the sample sites, seropositive provinces for *Anaplasma* spp. were Gyeongsangbuk-do (100%, 2/2), Jeollanam-do (50.0%, 4/8), Jeollabuk-do (36.8%, 7/19), Gyeongsangnam-do (26.7%, 4/15), Chungcheongnam-do (20.0%, 2/10), Gangwon-do (14.3, 1/7), and Gyeonggi-do (5.7%, 8/140), along with Ulsan (33.3%, 3/9), Daejeon (28.6%, 2/7), Busan (6.7%, 1/15), and Seoul (5.5%, 8/146) metropolitan cities (**Fig. 1**).

B. burgdorferi was identified in 12/430 dogs (2.8%). The *B. burgdorferi* seropositive dogs were from Gyeongsangnam-do (20.0%, 3/15), Jeollabuk-do (5.3%, 1/19), and Gyeonggi-do (1.4%, 2/140) provinces, and from the metropolitan cities of Busan (6.7%, 1/15) and Seoul (3.4%, 5/146) (**Fig. 1**).

E. canis had a lower seroprevalence rate and was identified in only 6/430 (1.4%) dogs. Gyeongsangnam-do and Gyeonggi-do were the seropositive provinces, along with Busan and Seoul metropolitan cities, with a seroprevalence rate of 6.7%, 0.7%, 13.3%, and 1.4%, respectively (**Fig. 1**).

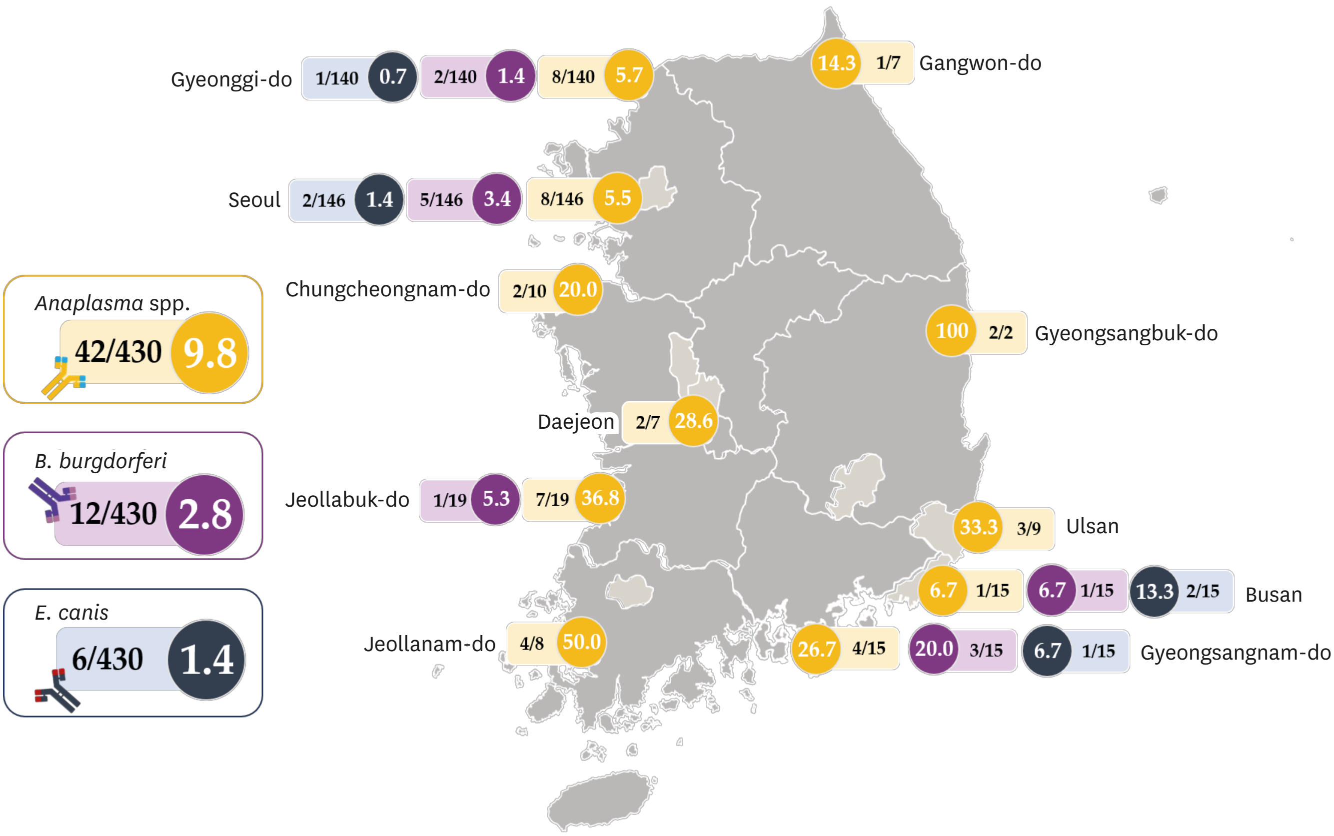

Fig. 1. Map illustrating the distribution of seropositive dogs to *Anaplasma* spp., *B. burgdorferi* and *E. canis* in 2019–2020, the Republic of Korea. Infection rates are shown in circles: yellow color for *Anaplasma* spp., purple for *B. burgdorferi*, and blue for *E. canis*.

Of the 430 dogs, seven (1.6%) were co-detected with double and triple antibodies. The types of co-infections found were *Anaplasma* spp. plus *B. burgdorferi* in three dogs (0.7%), *Anaplasma* spp. plus *E. canis* in one dog (0.2%), *B. burgdorferi* plus *E. canis* in one dog (0.2%), and a triple co-infection with *Anaplasma* spp., *B. burgdorferi*, and *E. canis* in two dogs (0.5%).

According to the gender classification, male dogs had a relatively high seroprevalence than female dogs: 10.4% vs. 8.5% for *Anaplasma* spp., 3.3% vs. 2.4% for *B. burgdorferi*, and 1.6% vs. 0.5% for *E. canis*. On the other hand, Chi-squared analysis showed no significant relationship between gender and the capacity to produce antibodies (**Table 2**). Based on the age groups, dogs between eight and 10 years old showed the highest seroprevalence rate of *Anaplasma* spp. in (10.6%, 7/66), whereas dogs between five and seven years old were the most seroreactive to *B. burgdorferi* and *E. canis*, with rates of 4.1% (4/97) and 2.1% (2/97), respectively. As shown in **Table 2**, these differences were not significant. On the other hand, when evaluating the risk factor "tick exposure" to seroprevalence, a significant difference was observed only for *Anaplasma* spp. (*A. phagocytophilum*/*A. platys*) with a *p* value of 0.047 (**Table 2**).

DISCUSSION

There is global concern regarding the growing spectrum of tick-borne diseases affecting animals and humans [15]. Therefore, the ROK has been making constant efforts to investigate the emergence and re-emergence of such diseases. Companion animals play a key

Table 2. Evaluation of the risk factors that may be associated with the presence of antibodies against *Anaplasma* spp., *B. burgdorferi*, and *E. canis* in companion dogs from the Republic of Korea in 2019–2020

Risk factor	No. tested	*Anaplasma* spp.			*B. burgdorferi*			*E. canis*		
		No.	IR %	*p* value	No.	IR %	*p* value	No.	IR %	*p* value
Sex				0.540			0.579			0.247
Male	182	19	10.4		6	3.3		3	1.6	
Female	211	18	8.5		5	2.4		1	0.5	
Unknown	37	5	13.5		1	2.7		2	5.4	
Total	430	42	9.8		12	2.8		6	1.4	
Age				0.832			0.334			0.834
≤ 1 year old	57	4	7		1	1.8		1	1.8	
2–4 years old	119	12	10.1		6	5		1	1	
5–7 years old	97	10	10.3		4	4.1		2	2.1	
8–10 years old	66	7	10.6		0	0		1	1.5	
≥ 11 years old	52	3	5.8		1	1.9		0	0	
Unknown	39	6	15.4		0	0		1	2.6	
Total	430	42	9.8		12	2.8		6	1.4	
Tick exposure				0.047*			0.112			0.140
Yes	222	24	10.8		8	3.6		5	2.3	
No	68	2	2.94		0	0		0	0	
Unknown	140	16	11.4		4	2.9		1	0.7	
Total	430	42	9.8		12	2.8		6	1.4	

IR, infection rate.
*Statistically significant.

role by acting as competent reservoirs and sentinels. The latter is one of the major concerns for the Korean population because the country has seen a steady increase in the companion animal population over the years [16].

Regarding *Anaplasma* species infecting Korean domestic dogs, only *A. phagocytophilum* has been identified by PCR with an extremely low prevalence, ranging from 0.1% to 2.3% [17,18]. Serological techniques, such as ELISA and IFA, have revealed a seroprevalence of *A. phagocytophilum* ranging from 15.1% to 26.4% [16,18-20]. These rates are slightly higher than those obtained in the present study using the SensPERT Ab Test Kit (9.8%). Interestingly, a different pattern of *Anaplasma* infections has been observed in China, where in addition to *A. phagocytophilum*, dogs have also been infected by *A. ovis*, *A. bovis* [21], and *A. capra* [22] species. Hence, continued epidemiological surveillance of these pathogens in the ROK is recommended because different genetic variants of zoonotic *A. capra* have recently been identified in Korean cattle [23].

B. burgdorferi in the ROK has only been identified by serology (ELISA and IFA), despite being evaluated by real-time PCR [18]. The seroprevalence rates reported over the years were 2.2% (5/229) in 2010, 1.1% (2/182) in 2012, and 1.1% (6/532) in 2017 [18-20]. The current study revealed a seroprevalence rate of 2.8% (12/430), which is similar to that identified in 2010 [20]. On the other hand, a serosurvey conducted in 2020, with a larger study population, identified a seroprevalence of 6.4% (142/2,215), which is the highest seroprevalence rate of *B. burgdorferi* reported thus far in the ROK [16]. China and Japan, the two close neighboring countries of the ROK, have also identified seroreactive dogs to this spirochete. Japan has the highest seroprevalence rate, ranging from 10.2% to 27.3% [24,25].

Regarding *Ehrlichia* species, two causative agents of canine ehrlichiosis have been identified in the ROK, *E. chaffeensis* and *E. canis*. In 2008, there were two clinical cases of canine ehrlichiosis caused by *E. chaffeensis*. One of the two dogs was also infected with *Babesia gibsoni*, another emerging tick-borne pathogen in the ROK [26]. In 2020, the seroprevalence of *E. chaffeensis* was 2.3% [16]. On the other hand, the seroprevalence of *E. canis* in this country ranged from 0 to 22.5% between 2010 to 2017 [18-20,27]. This pattern is consistent with the seroprevalence identified in the present study (1.4%), which is within the above-mentioned range. In China and Japan, the seroprevalence of *Ehrlichia* spp. has been low, only 1.3% and 1.5%, respectively [24,28].

Infection with multiple tick-transmitted pathogens or with multiple genotypes of the same pathogenic species can also occur in an individual animal after heavy exposure to ticks [29]. In the current study, of the different types of co-infections, *Anaplasma* spp. plus *B. burgdorferi* was identified as being the predominant combination. One study performed in Minnesota, USA, found an association between concurrent *A. phagocytophilum* and *B. burgdorferi* seroreactivity and clinical illness in dogs. Dogs testing positive for antibodies to both pathogens, *A. phagocytophilum* and *B. burgdorferi*, were almost twice as likely to have clinical signs consistent with anaplasmosis or borreliosis compared to dogs that were seroreactive to only one of these organisms [30]. Although the present investigation already demonstrated that co-infections with *Anaplasma* spp. and *B. burgdorferi* are also occurring in the ROK, further studies will be needed to clarify the relationship between the seroreactivity and the clinical presentation in the country.

There was no statistical significance in any of the risk factors evaluated in this study, such as sex, age, and history of tick exposure that could be associated with the presence of antibodies against *Anaplasma* spp., *B. burgdorferi*, and *E. canis*. There was only one exception: a case of *Anaplasma* spp., where the risk factor "tick exposure" was statistically significant ($p = 0.047$). In the ROK, *Haemaphysalis longicornis*, *Ixodes persulcatus*, and *Ixodes turdus* ticks have been identified as potential vectors of *A. phagocytophilum* [31]. Moreover, according to a tick surveillance conducted in 2011, *H. longicornis* (48.9%), *Haemaphysalis flava* (17.3%), *Ixodes nipponensis* (1.7%), and *Rhipicephalus sanguineus* sensu lato (0.5%) were the most prevalent tick species infesting Korean dogs [32]. Among the tick species mentioned above, *H. longicornis* is distributed widely throughout the ROK [33]. This tick is the main vector for severe fever with thrombocytopenia syndrome virus (SFTSV), a new tick-viral zoonosis in East Asian countries, including China, Japan, and the ROK [34]. The most recent seroprevalence of SFTSV identified in Korean dogs was 21.4%. On the other hand, in humans, after the first clinical case was identified in 2003, 335 cases (73 deaths; case fatality rate 21.8%) were reported from 2013 to 2016 [35]. These findings, together with the above results, reinforce the importance of continued epidemiological surveillance of tick-borne pathogens in the ROK because many of them have zoonotic potential. Furthermore, the serological survey has shed new light not only on the geographic distribution and seroprevalence rates of *Anaplasma* spp., *B. burgdorferi*, and *E. canis* in dogs but also in the use of the SensPERT One-rapid test kit (VetAll Laboratories, Korea) as a useful tool for identifying seroreactive animals.

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