Molecular Detection of *Anaplasma*, *Bartonella*, and *Borrelia theileri* in Raccoon Dogs (*Nyctereutes procyonoides*) in Korea

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Abstract. Anaplasmosis, cat-scratch disease, and Lyme disease are emerging vector-borne infectious diseases in Korea. Although the prevalence of vector-borne pathogens (VBPs) in domestic animals and vector arthropods has been documented, there is limited information on the presence of VBPs in wild animals. The raccoon dog (*Nyctereutes procyonoides*), a wild canid found in East Asia and Europe, represents a potential wildlife reservoir for zoonotic diseases. To investigate the prevalence of VBPs in raccoon dogs, 142 carcasses and 51 blood samples from captured raccoon dogs were collected from 2003 to 2010 and from 2008 to 2009, respectively, in Korea. In addition, 105 *Haemaphysalis flava* (14 larvae, 43 nymphs, 32 males, and 16 females) and nine *Haemaphysalis longicornis* (all female) were collected from three raccoon dogs. Samples of the spleen and blood were tested for the presence of VBPs by using nested polymerase chain reaction. Among the samples collected from 193 raccoon dogs and 114 ticks, two samples were positive for *Anaplasma phagocytophilum*, four for *Anaplasma bovis*, two for *Borrelia theileri*, and two for *Bartonella henselae*. To the best of our knowledge, this study is the largest survey of raccoon dogs aimed at the analysis of VBPs in this species. Moreover, the present study represents the first identification of *A. phagocytophilum*, *B. henselae*, and *B. theileri* in raccoon dogs in their native habitat (East Asia).

INTRODUCTION

The raccoon dog (*Nyctereutes procyonoides*) is a species native to East Asia (East Siberia, East Mongolia, China, North Vietnam, Japan, and Korea), but was introduced into the European fauna in the twentieth century for fur production.¹ The typical habitat of raccoon dogs includes forested river valleys, surrounding lakes, and reed beds. The particular behavioral characteristics of raccoon dogs, such as the habitation of burrows constructed by other animals or their own, exposes them to contact with ectoparasites infected with several pathogens.¹ Recently, in Korea, the population of raccoon dogs has increased significantly owing to the absence of natural predators and their high adaptability to diverse environments.² This is important as the raccoon dog plays a central role in the circulation of rabies in Korea.^{2,3} Moreover, the prevalence of tick-borne pathogens that originate from raccoon dogs has been reported in Europe, Japan, and Korea.^{4–7} Therefore, the increased population of raccoon dogs, and the resulting elevated zoonotic disease risk, represents a potential public health threat.

Anaplasma phagocytophilum is a zoonotic intracellular bacterium transmitted by ticks of the *Ixodes* and *Haemaphysalis* genera in the United States, Europe, and Asia.^{8–10} This bacterium infects various hosts worldwide, including humans, ruminants, horses, dogs, cats, birds, deer, and rodents.¹¹ Recently, in Korea, *A. phagocytophilum* DNA has been detected in several mammals such as deer, rodents, goats,

dogs, and cats.^{9,12–16} Previous reports have indicated that *A. phagocytophilum* is widely circulating among wildlife systems and may be transmitted to humans and companion animals. Accordingly, the first isolation of *A. phagocytophilum* from a Korean resident has been recently reported.¹⁷

Bartonella henselae, a gram-negative facultative intracellular bacillus transmitted by the cat flea (*Ctenocephalides felis*), causes cat-scratch disease.¹⁸ Although fleas are known to be the main vector of *Bartonella* spp., *Bartonella* DNA has recently been detected in ticks and other animals in Korea, including Korean water deer (KWD, *Hydropotes inermis argyropus*), dogs, leeches, and cats.^{9,13,19–23} A recent study showed that *Ixodes* spp. are capable of transmitting *B. henselae* via salivary contents,²⁴ suggesting that the reservoir/host range of *Bartonella* spp. could include wildlife. In addition, the first isolation of *B. henselae* from humans was reported in Korea²⁵ and the first detection of *Bartonella* spp. was reported in raccoon dogs in Japan.⁶

Borrelia spp. are generally classified into the Lyme disease group and relapsing fever group.^{26,27} The Lyme disease group is associated with many *Borrelia* spp. such as *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, etc., and are transmitted by hard ticks (*Ixodidae*).^{26,27} On the other hand, the relapsing fever group of *Borrelia* spp., such as *Borrelia duttonii* and *Borrelia crocidurae*, are transmitted by soft ticks (*Argasidae*).²⁸ However, some members of the relapsing fever group, including *Borrelia theileri*, *Borrelia miyamotoi*, and *B. lonestari* are transmitted mainly by hard ticks (*Ixodidae*).^{29–31} In Korea, *B. afzelii* has been isolated from *Ixodes* spp. and the DNAs of *Borrelia turdi* and unidentified *Borrelia* spp. have been detected in *Haemaphysalis* ticks collected from migratory birds and goats.^{13,19,32} Moreover, the serological prevalence of Lyme disease in horses and dogs has also been reported.^{33,34} However, information detailing the prevalence of *Borrelia* spp. in animals is still lacking.

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In Korea, *Haemaphysalis*, the predominant genus of ticks found in grasses/herbaceous vegetation habitats and wild/domestic animals, is infected with numerous vector-borne pathogens (VBPs).^{9,10,19,20} According to a previous study, VBPs are endemic, present in ticks collected from deer in Korea.^{12,20,22} These reports indicate that wild animals may represent additional reservoirs for VBPs. However, the prevalence of vector-borne agents has not been reported in populations of wild animals in Korea (other than in KWD). Here, we describe the prevalence of VBPs in raccoon dogs in Korea. This will have implications for the future control of such pathogens in East Asia.

MATERIALS AND METHODS

Ethical statement. All samples were collected with consent from the Conservation Genome Resource Bank (CGRB) for Korean Wildlife. This study was approved by the Institutional Committee of Graduate studies and Research at Seoul National University.

Sample collection. The carcasses of raccoon dogs were collected from eight provinces in Korea, between 2003 and 2010, by the CGRB for Korean Wildlife. Carcasses were stored at -20°C until necropsy for harvesting splenic tissues. Small sections of splenic tissue were frozen at -80°C until DNA extraction.

Live raccoon dogs were captured using traps in three provinces for a rabies bait vaccine program in Korea between 2008 and 2009. Captured raccoon dogs were anesthetized by intramuscular injection of 2 mg/kg of xylazine (Bayer Korea Ltd., Seoul, Korea) and 10 mg/kg of ketamine (Yuhan Corporation, Seoul, Korea). After anesthesia, blood was harvested from the jugular vein and stored in ethylenediaminetetraacetic acid-containing tube. All samples were stored at 4°C until DNA extraction. Ticks were collected from the captured raccoon dogs by placing fine tweezers around the mouth part, slowly removing the attached tick, and then storing them in vials containing 70% ethanol. The ticks were transported to the laboratory and identified microscopically to assess the stage of development and species, according to previously reported guidelines.³⁵

DNA extraction and polymerase chain reaction (PCR) amplification. DNA was extracted from spleen and blood samples using DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For DNA extraction, 10 mg of splenic tissue and 100 μL of blood were used. Conventional and nested PCRs were performed using primers specific for *A. phagocytophilum* (16S rRNA, *groEL*, *msp2*, and *ankA* genes), *Anaplasma bovis* (16S rRNA), *Bartonella* (16S-23S internal transcribed spacer [ITS], *rpoB*, and *groEL*), and *Borrelia* spp. (16S rRNA and *groEL*), as described in previous studies.^{12,13,36–38} A molecular identification of tick species was conducted to confirm the visual assessment of tick species, using ITS2 and 16S rRNA primer sets.³⁹ In raccoon dogs, mitochondrial cytochrome b (mt *cyt b*) and c (mt *cyt c*) genes were selected as conserved genes to prevent false-negative results in the PCR. The primer set used to amplify mt *cyt b* was a universal primer set specific for mammalian hosts.⁴⁰ The primer used to amplify mt *cyt c* was specific for raccoon dogs.⁴¹ *Anaplasma phagocytophilum* (genomic DNA from the Webster strain, provided by J. Stephen Dumler [Johns Hopkins University School of Medicine, Baltimore, MD]) was used as a positive control for

detecting *Anaplasma* spp.; *B. henselae* (Houston-1 strain, ATCC[®] 49882[™]) and *B. burgdorferi* sensu stricto (297 strain, ATCC 53899[™]) isolates were purchased from the American Type Culture Collection and used as positive controls. The first and nested PCRs were performed in a total volume of 25 μL . Each PCR mixture consisted of 10 pmol of primers, 1 U of recombinant Taq DNA polymerase (Takara Bio, Inc., Koyto, Japan), 10 $\times$ PCR buffer (Takara Bio, Inc.), 2.5 mM deoxynucleotide solution mixture (Takara Bio, Inc.), 1 μL samples of genomic DNA for the first PCR, and 1 μL of the first PCR product for the second PCR. Amplification of PCR products was performed using a SimpliAmp[™] thermal cycler (Thermo Fisher Scientific Inc., Singapore), and PCR conditions, as previously described.^{12,13} The PCR amplicons were visualized by gel electrophoresis using a 1.2% agarose gel.

Nucleotide sequencing and phylogenetic analysis. The PCR products were purified using QIAquick Gel Extraction kits (Qiagen). All positive PCR amplicons were directly sequenced on an ABI prism 3730 DNA sequence analyzer (Applied Biosystems, Foster City, CA). The obtained sequences were evaluated using Chromas software (Ver 2.33; Technelysium Pty Ltd, South Brisbane, Australia), aligned using the Clustal X algorithm (Ver 2.1; Conway Institute, University College Dublin, Dublin, Ireland), and then analyzed with a similarity matrix. Relationships between individuals were assessed using a maximum likelihood method with nucleotide distance (Kimura 2 parameter) with 1,000 replications for a bootstrap test. The phylogenetic trees of nucleotide sequences (547-bp 16S rRNA gene of *Anaplasma*, 507-bp 16S rRNA gene of *Borrelia*, 467-bp *msp2* gene of *Anaplasma* (b), 460-bp *ankA* gene of *Anaplasma*, and 569-bp ITS gene of *Bartonella*) were constructed using the MEGA7 program (<http://www.megasoftware.net/>). GenBank accession numbers of the 16S rRNA, *msp2*, *ankA*, and ITS region sequences, and specific genospecies sequences related to pathogens for sequence comparisons are included in Figures 1–3.

RESULTS

A total of 193 raccoon dogs and 114 ticks were used for this study. Splenic tissue samples were collected from 152 carcasses in eight provinces (20 from Seoul, 40 from Gyeonggi-do, 41 from Gwangwon-do, four from Chungcheongbuk-do, two from Chungcheongnam-do, 15 from Jeollanam-do, one from Gyeongsangbuk-do, 12 from Gyeongsangnam-do, and seven from unknown) (Table 1). Blood samples from 51 captured raccoon dogs were collected in three provinces (three from Seoul, 12 from Gyeonggi-do, 31 from Gwangwon-do, and five from unknown) (Table 1). A further 114 ticks, including 105 belonging to the *Haemaphysalis flava* species (14 larvae, 43 nymphs, 32 males, and 16 females) and nine of the *Haemaphysalis longicornis* species (all females), were collected from three captured raccoon dogs (from Gwangwon-do). However, blood samples were not collected from these raccoon dogs (Table 2).

A molecular differentiation of tick species between *H. flava* and *H. longicornis* was conducted by PCR and sequencing to confirm the morphological identification of tick species. All raccoon dog samples were amplified using *cyt b* and *cyt c* primer sets. Amplified PCR products were randomly selected and sequenced, confirming they were raccoon dog sequences. The obtained *cyt b* and *cyt c* sequences were identical to the previously reported *Nyctereutes procyonoides*

FIGURE 1. Phylogenetic relationships for *Anaplasma phagocytophilum* (bold letters) and *Anaplasma bovis* (bold letters) detected from raccoon dogs and *Anaplasma* and *Ehrlichia* species based on partial nucleotide sequences of 547-bp 16S rRNA gene (A), 467-bp *msp2* gene (B), and 460-bp *ankA* gene (C) fragments. The maximum likelihood method was used for constructing the phylogenetic tree. The numbers at the nodes are the proportions of 1,000 bootstrap iterations that support the topology shown.

koreensis sequences, JX099864 and KF709435, respectively. In addition, all tick samples were amplified using ITS2 and 16S rRNA primers. The obtained *H. flava* and *H. longicornis* sequences were identical to a previously reported *H. flava* sequence (AB861941) and *H. longicornis* sequence (AB819210), respectively (data not shown). These results indicated that the morphological keys used for visual assessment were accurate.

Anaplasma phagocytophilum and *A. bovis* 16S rRNA genes, of product sizes 926 bp and 547-bp, respectively, were detected by species-specific nested PCR. *Anaplasma phagocytophilum* was detected in one (RS10556) of the 142 spleen samples and one (RB3) of the 51 blood samples (Table 1). The obtained sequences shared 99.6% identity with each other. The RB3 sequence (KY458571) was identical to the *A. phagocytophilum* HZ strain sequence (CP000235), Korean partial sequence (GU064899) detected in *H. longicornis*, and a partial sequence (JX173652) from Austria (Figure 1A). The RS10556 sequence (KY458570) was 99.9% identical to a Korean partial sequence (KU513794) detected in a sample from a dog. *Anaplasma bovis* 16 rRNA gene sequences were detected in one (RS7075) of the 142 spleen samples and in three (RB9, RB10, and RB28) of the 51 blood samples

(Table 1). The gene sequences were compared with 16S rRNA gene sequence fragments to elucidate the genetic relationships between the *Anaplasma* spp. identified in raccoon dogs. The obtained *A. bovis* sequences were between 98.7% and 100.0% identical to each other and between 98.7% and 99.8% similar to a previously reported *A. bovis* sequence (GU556627) detected in a deer in Korea (Figure 1A). In comparison, the similarities between the obtained sequences and a previously reported *A. bovis* sequence (GU937020) collected from a raccoon, the most morphologically similar animal to the raccoon dog, were reported to be between 98.4% and 99.5%.

Using an *msp2* and *ankA* gene-specific nested PCR, one *A. phagocytophilum* *msp2* gene sequence and one *A. phagocytophilum* *ankA* gene sequence (MF506839) were obtained from the spleen samples. The *A. phagocytophilum* *msp2* gene sequence (MF506840) was identical to an *A. phagocytophilum* sequence (HM752099) from Korea and 97.2% similar to several other *A. phagocytophilum* strains (MRK, Dog2, HZ, and JM) (Figure 1B). The *A. phagocytophilum* *ankA* gene sequence was identical to the Korean partial sequence (HM752100) detected from a KWD and was 98.7%

FIGURE 2. Phylogenetic relationships for *Bartonella henselae* (bold letters) detected from raccoon dogs and *Bartonella* spp. based on partial nucleotide sequences of internal transcribed spacer gene fragments (569-bp). The maximum likelihood method was used for constructing the phylogenetic tree. The numbers at the nodes are the proportions of 1,000 bootstrap iterations that support the topology shown.

similar to a previously reported *A. phagocytophilum* sequence (AY502606) from Russia (Figure 1C).

Two *B. henselae* sequences were detected in the spleen samples of raccoon dogs in Korea, via an ITS-specific nested PCR (Table 1). The *B. henselae* sequences were not detected in the blood samples. The size of the obtained product was 569-bp and contained a noncoding region. The two obtained *B. henselae* sequences (KY464064 and KY464065) were identical to each other and matched the *B. henselae* Houston-1 strain (BX897699), URBHLIE9 strain (AF312496), and a Korean partial sequence (KC422265). In addition, the sequences were 99.8% similar to a previously reported *B. henselae* sequence (JQ009430) detected in dogs in China (Figure 2). However, the nested PCR targeting *rpoB* and *groEL* genes that was performed for additional phylogenetic analysis did not yield any positive PCR products.

The *B. theileri* 16S rRNA gene was also detected by nested PCR, with an amplicon size of 507-bp. Two *B. theileri* sequences were identified from the spleen sample from a raccoon dog and a female *H. flava* (Tables 1 and 2). The obtained *B. theileri* sequences (KY458576 and KY798215) were identical to each other and showed 99.8% homology to a *Borrelia* sp. sequence (AB897891) from Japan, 99.6% homology to a *B. theileri* sequence (KF569941) from Mali, and 99.6% homology to a *Borrelia* sp. sequence (KC422269) from Korea.

However, phylogenetic analysis indicated that KC422269 and KF569941 were more closely related to the obtained sequence than AB897891 (Figure 3). In addition, KC422269 (the obtained sequence from *H. longicornis* collected from goats in North Korea) appeared to be *B. theileri*, based on phylogenetic analysis (Figure 3). However, no PCR amplicons were obtained from the *groEL* gene-specific nested PCR.

In summary, two *A. phagocytophilum*, four *A. bovis*, two *B. theileri*, and two *B. henselae* sequences were detected by nested PCR in 193 raccoon dogs and 114 ticks (Tables 1 and 2). None of the raccoon dogs or ticks analyzed showed dual-pathogen co-infection.

DISCUSSION

Over the last decade, the investigation of VBPs in Korea has mainly focused on domestic animals, rodents, and vectors.^{9,10,14-16,42,43} However, recent studies examining wild animals have indicated that VBPs are prevalent in wild/domestic animals and vectors, and that the occurrence of human vector-borne diseases in Korea may, therefore, be more likely.^{12,22} Consistent with these findings, the first isolation of *B. henselae* and *A. phagocytophilum* from humans has been reported, and the reemergence of Lyme disease in Korea represents a potential public health concern.^{17,25,44}

FIGURE 3. Phylogenetic relationships for *Borrelia theileri* (bold letters) detected from raccoon dogs and *Borrelia* spp. based on partial nucleotide sequences of 16S rRNA gene fragments (507-bp). The maximum likelihood method was used for constructing the phylogenetic tree. The numbers at the nodes are the proportions of 1,000 bootstrap iterations that support the topology shown.

The aim of the present study was to investigate the role that raccoon dogs may play in the enzootic maintenance of VBPs in Korea. To the best of our knowledge, this study is the largest survey of such pathogens in raccoon dogs. The sampling period was over 8 years and 193 raccoon dogs were studied in total. Importantly, the present work constitutes the first report

of the prevalence of *A. phagocytophilum*, *B. henselae*, and *B. theileri* in raccoon dogs in their East Asian native habitat.

To date, studies of the occurrence of *A. phagocytophilum* in wild animals in Asia have focused mainly on deer. For example, *A. phagocytophilum* and *A. bovis* have been detected in KWD and sika deer (*Cervus nippon*) in Korea and Japan.^{12,45}

TABLE 1
Detection of vector-borne pathogens from the spleen and blood of raccoon dogs in Korea during 2003–2010

Region	Spleen					Blood		
	Number of samples	Number of PCR-positive samples (infection rate [%])				Number of samples	Number of PCR-positive samples (infection rate [%])	
		<i>Anaplasma phagocytophilum</i>	<i>Abovis bovis</i>	<i>Borrelia</i> sp.	<i>Bartonella henselae</i>		<i>A. phagocytophilum</i>	<i>A. bovis</i>
Seoul	20	0	0	0	0	3	1 (33.3)	0
Gyeonggi-do	40	0	0	0	1 (2.5)	12	0	0
Gwangwon-do	41	1 (2.4)	1 (2.4)	0	0	31	0	3 (9.7)
Chungcheongbuk-do	4	0	0	0	0	—	—	—
Chungcheongnam-do	2	0	0	0	0	—	—	—
Jeollanam-do	15	0	0	1 (6.6)	0	—	—	—
Gyeongsangbuk-do	1	0	0	0	0	—	—	—
Gyeongsangnam-do	12	0	0	0	0	—	—	—
Unknown	7	0	0	0	1 (14.3)	5	0	0
Total	142	1 (0.7)	1 (0.7)	1 (0.7)	2 (1.4)	51	1 (2.0)	3 (5.9)

PCR = polymerase chain reaction.

TABLE 2

Prevalence of vector-borne pathogens in ticks collected from raccoon dogs in ROK during 2008–2009

Species	Stage	Number of pools (number of ticks)	Number of PCR-positive samples (prevalence [%]) against <i>Borrelia</i> sp.
<i>Haemaphysalis flava</i>	Larva	2 (14)	0
	Nymph	9 (43)	0
	Male	32 (32)	0
	Female	16 (16)	1 (6.3)
	Subtotal	59 (105)	1 (1.0)
<i>Haemaphysalis longicornis</i>	Female	9 (9)	0
	Subtotal	9 (9)	0
	Total	68 (114)	1 (0.9)

PCR = polymerase chain reaction.

* PCR-positive pathogens were calculated by minimum infection rate and number of positive pools/total number of individual ticks tested.

Very recently, *A. bovis* was detected in raccoon dogs in Korea.⁶ In Europe, the molecular detection of *A. phagocytophilum* in raccoon dogs by real-time PCR based on the *msp2* gene has been attempted, although gene sequences matching those of *A. phagocytophilum* were not obtained.⁴ In this study, the 16S rRNA gene sequences of *A. phagocytophilum* (1.0%, 2/193) and *A. bovis* (2.1%, 4/193) were successfully detected in both spleen and blood samples from raccoon dogs sampled in Korea. This study is, therefore, the first to report the molecular detection of *A. phagocytophilum* DNA in raccoon dogs. The acquired 16S rRNA sequences of *A. phagocytophilum* and *A. bovis* were either identical or similar to previously reported sequences detected in wild rodents, deer, and ticks in Korea (Figure 1A). Additional phylogenetic analysis of *ankA*, *msp2*, and *groEL* genes via PCR was also performed. However, only one *ankA* (MF506839) and one *msp2* (MF506840) of *A. phagocytophilum* sequence were amplified from a single splenic tissue (Figure 1B and C). These data suggest that the prevalence of *A. phagocytophilum* in raccoon dogs is lower than that in KWD (although only limited studies on the prevalence of *Anaplasma* spp. in raccoon dogs are available) and indicate that raccoon dogs may play a role in the dispersal or maintenance of *A. phagocytophilum* in Korea. Further studies are needed to clarify the role that species plays in the ecology of *A. phagocytophilum*.

The molecular detection of *Bartonella* spp. in raccoon dogs has also been reported in Japan and their sequences were found to be most closely related to those of *Bartonella rochalimae*.⁷ In addition, *Bartonella washonensis* and other *Bartonella* spp. were detected in carnivores of the suborder Caniformia in Japan.⁷ In the present study, the obtained ITS gene sequences from raccoon dogs were identified as similar to those of *B. henselae*, a species transmitted by the cat flea (*C. felis*).¹⁸ Although *B. rochalimae* (genetically similar to *Bartonella clarridgeiae* and lineage three of the *Bartonella* genus) was detected in raccoon dogs, the detection of *B. henselae* (lineage four of the *Bartonella* genus) in this study is a novel discovery.⁴⁶

To our knowledge, *B. theileri* has primarily been detected in ungulate mammals and hard ticks.^{31,47–49} However, *B. theileri* DNA was detected in raccoon dogs in this study, which is the first identification of this species in a carnivore rather than in an ungulate species. Although this result indicates that *B. theileri* can infect raccoon dogs, it also suggests that further studies

are needed to clarify the role that the species may have as a potential reservoir of *B. theileri*. In addition, one *B. theileri* sequence was detected from a *H. flava* tick. We found 105 *H. flava* and nine *H. longicornis* sequences collected from three raccoon dogs demonstrating that *H. flava* is the dominant species found on raccoon dogs. Although the AB897891 sequence (detected from a *Haemaphysalis japonica* tick in Japan) and KC422269 sequence (detected from a *H. longicornis* tick in North Korea) were not categorically identified as belonging to *B. theileri*, they were included in the same sub-clade with KF569941, KY458576, KY798215, and U38375, based on phylogenetic analysis (Figure 3). We, therefore, suggest that the AB897891 and KC422269 sequences could potentially belong to *B. theileri*. Although *Rhipicephalus* spp. are known to be the main vector for *B. theileri*, our studies indicate that *Haemaphysalis* ticks may be additional vectors of *B. theileri* in East Asia.¹⁷

In one previous study, 117 *H. flava* and 18 *Ixodes tanuki* were collected from one raccoon dog.⁵⁰ Moreover, in another previous study, 105 *H. flava* and nine *H. longicornis* were collected from three raccoon dogs.⁵¹ Although these previous studies, and our own results, are not representative of the total tick population in raccoon dogs in Korea, they indicate that *H. flava* is the dominant tick species found on raccoon dogs in the country. It has in addition been shown that *H. longicornis* represents the dominant tick species found on domestic/wild animals that live in grasses in Korea.^{9,10,13,19,20} To fully understand the differences in the tick populations between raccoon dogs and other animals, further study is required.

Ixodes spp. are the main vectors for both anaplasmosis and Lyme borreliosis.^{8,27} However, *Haemaphysalis* spp. in addition act as a vector for the transmission of *Anaplasma* spp., and possibly *Bartonella* and *Borrelia* spp., in Korea.^{9,10,13,19,20} In particular, although *Bartonella* spp. are mainly transmitted by fleas, recent evidence demonstrating transmission by ticks has been reported.^{11,17,18,22} Moreover, the rapid expansion of reservoir-adapted pathogens, such as *Anaplasma*, *Bartonella*, and *Borrelia* spp., has been described.^{4,5,12,13,22,23,52} These previous studies and our present work suggest that raccoon dogs may serve as potential reservoirs for the transmission of VBPs. Furthermore, the increased population of wild animals may contribute to the transmission of VBPs between wildlife, humans, and companion animals in Korea. These findings indicate that further investigation is required to fully understand the tick populations present within the raccoon dog population and elucidate the distribution of ectoparasites and VBPs in wild animals in Korea.

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