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Comparison of canine vector-borne diseases in rural dogs based on the prevention status

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Abstract

Canine vector-borne diseases (CVBDs) are transmitted by different groups of hematophagous arthropod vectors that are distributed worldwide and can cause significant health problems for dogs. The aim of this study was to investigate and compare the prevalence of selected CVBD pathogens in rural outdoor dogs based on prevention status. Between June 2017 and February 2019, blood samples were collected from 343 clinically healthy rural dogs composing two different groups: systematically managed dogs (SMD; n=92) and personally managed dogs (PMD; n=251). Vaccination and preventive medications were applied strictly following the programmed schedule for the SMD group; in contrast, in the PMD group, they were applied only when requested by the dog owners. Serological and molecular assessments showed that significantly more dogs in the PMD group were infected with *B. gibsoni* ($P<0.001$) and *D. immitis* (P=0.001) than those in the SMD group. These findings suggest that the regular use of preventive medications and environmental controlling efforts contribute to reducing the prevalence of CVBD pathogen infections. In addition, dogs infected with certain kinds of CVBD pathogens could remain asymptomatic, suggesting that continuous monitoring and periodic preventive treatment should be conducted even for clinically healthy dogs.

Key words : Canine vector-borne disease, Preventive medicine, *Babesia gibsoni*, *Dirofilaria immitis*, *Mycoplasma haemocanis*

INTRODUCTION

Canine vector-borne disease (CVBD) pathogens, such as *Anaplasma phagocytophilum*, *Babesia gibsoni*, *Dirofilaria immitis*, *Hepatozoon canis*, and *Mycoplasma haemocanis*, cause serious illness in dogs worldwide. These pathogens are transmitted by hematophagous arthropod vectors, mosquitoes, fleas, and tick species, in addition to direct blood contamination, such as through blood transfusions or bite wounds from other infected animals (Birkenheuer et al, 2005; Jefferies et al, 2007; Dantas-Torres et al, 2008).

Environmental factors such as vector distribution and populations, climate change, seasonal changes in temperature/humidity, and lifestyle of the host (indoor or outdoor) have been suggested to increase vector species, and accordingly CVBD pathogen, exposure opportunities (Genchi et al, 2009; Movilla et al, 2016; Aktas and Ozubek, 2018). Dogs can appear asymptomatic for several years following acute or sub-clinical infection with the pathogens (Boozer and Macintyre, 2005; Labarthe and Guerrero, 2005; Dantas-Torres et al, 2008; Baneth and Cohn, 2016; Aktas and Ozubek, 2018). In addition,

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infection with certain kinds of CVBD pathogens or low-burden infections also may remain inapparent during the prepatent period of pathogens in the body of dogs (Otranto et al, 2009; Lee et al, 2011; Baneth and Cohn 2016). Accordingly, the CVBD pathogen-infected dogs can serve as competent reservoir hosts of the pathogens and readily available of disease transmission for numerous types of blood-feeding arthropods, which is one of the most critical concerns in public health (Byun et al, 2007; Lee et al, 2010; Baneth and Cohn, 2016).

Preventive medications such as heartworm prophylaxis, chemophylaxis, and insecticides have been the most common treatments and are known to be highly effective in reducing the occurrence of CVBD (Labarthe and Guerrero, 2005; Lee et al, 2010; Ortranto et al, 2010; Stull et al, 2016). However, CVBD pathogen infection is reportedly associated with prevention status (Movilla et al, 2016; Suh et al, 2017; Aktas and Ozubek, 2018). In this regard, regularity and frequency of drug administration affect the prevention status against the infection (Boozer and Macintire, 2005; Goyena et al, 2016; Laidoudi et al, 2019). Additionally, environmental controlling efforts influence the prevalence of CVBD pathogen infection (Lee et al, 2010; Stull et al, 2016).

Most studies have focused on environmental risk factors for CVBD transmission and only emphasized on threats to public health. In addition, to date, little information has been available regarding the prevalence of CVBD pathogen infections according to different prevention statuses. The aim of this study was to investigate the prevalence of selected CVBD pathogens in rural outdoor dogs in the Republic of Korea (ROK) and compare the infection rates according to different management systems, especially prevention statuses. Our results should contribute to the development of better strategies for prevention of CVBD pathogen infections.

MATERIALS AND METHODS

Animals

Between June 2017 and February 2019, blood samples were collected from 360 clinically healthy dogs, weighing 15～28 kg, aged 6 months to 12 years. The dog-breeding farms were located in rural areas of Jindo-gun, Jeollanam-do province and Gyeongsan-si, Gyeongsangbuk-do province. All dogs were raised outdoors primarily for preserving Korean-native purebreds. The facilities were supervised by dog breed associations and/or local government. The two study groups consisted of personally managed dogs (PMD; *n*=251) and systematically managed dogs (SMD; *n*=92). This study was approved by Institutional Animal Care and Use Committee of the National Institute of Animal Science, Rural Development Administration in the ROK (Approval No.: 2017-257).

PMD group

Dogs in the PMD group were raised in outdoor barred cages, singly or sometimes grouped based on the decision of the dog owners. Vaccinations, wound treatments, health checkups, and preventive medications against heartworm and endo- and ectoparasites were provided by the local government upon request of the dog owners. The dog associations advised strict adherence to monthly application of the drugs between April and November every year; however, practical administration of the medications and the application schedule was owner-specific. Treatment against specific ectoparasite was performed once the parasites were observed.

SMD group

The SMD group consisted of dogs fully managed by a single organization supported by the local government. The dogs were raised in separate room-like kennels. Their health statuses were regularly monitored, and treatments were administered by employed veterinarians. Vaccinations, wound treatments, health checkups, and preventive medications against heartworm and other infectious diseases were provided strictly on schedule between April and November every year. Oral endoparasite preventive drug was administered once or more per two months for dogs below a year old and once per three months for dogs over one year of age. Treatment against specific ectoparasite was performed once the parasites were observed. In addition to providing treat-

ment, the organization controlled the surroundings by removing vegetation and cleaning the kennels every day.

Sample collection

Approximately 3 mL of whole blood was collected intravenously from each dog by an experienced veterinarian and no animal was harmed. Blood samples were collected into EDTA blood-collection tubes (Vacutainer K2 EDTA; Becton Dickinson, Bedford, MA, USA) and serum-collection tubes (Vacutainer SST II™; Becton Dickinson, USA). Sera samples were separated by centrifugation of whole blood for 15 min at 2095×*g* (Allegra® X-15R; Beckman Coulter, Brea, CA, USA).

Complete blood count (CBC) and serum biochemistry

CBC was measured using Procyte DX hematological analyzer (IDEXX, Westbrook, ME, USA). Serum biochemical profiling was conducted using VetLab® Station (IDEXX, USA) within 24 h from sample collection.

Rapid ELISA assay

Serum samples were tested for *Dirofilaria immitis* antigen and *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, and *Ehrlichia* spp. antibodies using a commercial rapid ELISA kit (SNAP® 4Dx® Plus; IDEXX, USA) according to the manufacturer's instructions.

PCR assay

DNA was extracted from whole blood samples and real-time PCR (Tick/Vector Comprehensive RealPCR™ Panel-Canine; IDEXX, USA) was performed at a commercial laboratory (Medexx, Seoul, Korea) to detect *A. phagocytophilum*, *A. platys*, *Babesia* spp., *Bartonella* spp., *Candidatus Mycoplasma haematoparvum*, *Mycoplasma haemocanis*, *Ehrlichia* spp., *Hepatozoon americanum*, *Hepatozoon canis*, *Leishmania* spp., *Neorickettsia risticii*, and *Rickettsia rickettsii*. Samples positive for *Babesia* spp. were submitted for species-specific PCR.

Statistical analysis

Data were entered into Excel 2010 (Microsoft®, Redmond, WA, USA) and statistically analyzed using SPSS software version 22.0 (IBM, Armonk, NY, US). The prevalence of the detected CVBD pathogens was tested for associations with management system and age and sex of the dogs in the PMD group using Fisher's exact test. P value < 0.05 was considered to indicate statistically significant differences.

RESULTS

CBC and blood chemistry results

All dogs in this study were considered healthy upon gross clinical examination, with CBC and biochemical profiles within the reference ranges. Additionally, no specific changes, such as PCV below the reference value or changes in terms of eosinophilia or liver-enzyme levels, were observed in the blood panels of CVBD-infected dogs.

Prevalence of CVBD pathogens in different management systems

In a total of 343 dogs, real-time PCR and serological analysis revealed CVBD pathogen infections in 89 dogs (25.9%) in both the SMD and PMD groups, which included 79 dogs infected with one pathogen and 10 dogs simultaneously infected with two pathogens (Table 1). In the SMD group, only six of the 92 dogs (6.5%) were infected with CVBD pathogens; namely, four were infected with *M. haemocanis* (4.3%) and two with *D. immitis* (2.2%). In the PMD group, 83 of the 251 dogs (33.1%) were infected with at least one CVBD pathogen, among which *B. gibsoni* (n=40, 15.9%) was the predominant, followed by *D. immitis* (n=36, 14.3%), *M. haemocanis* (n=14, 5.6%), *H. canis* (n=2, 0.8%), and *A. phagocytophilum* (n=1, 0.4%). Significant variations were observed in the prevalence of *D. immitis* (P=0.001) and *B. gibsoni* ($P < 0.001$) infections between the PMD and SMD groups. However, no significant differences were

Table 1. Prevalence of selected canine vector-borne disease pathogens in rural outdoor dogs according to management strategy

Category	Dogs examined *n* (%)	*D. immitis*		*M. haemocanis*		*B. gibsoni*		*A. phagocytophilum*		*H. canis*	
		No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)
Management											
SMD*	92 (26.8)	2 (2.2)	0～5.2	4 (4.3)	0.2～8.5	0 (0)	-	0 (0)	-	0 (0)	-
PMD	251 (73.2)	36 (14.3)	10～18.7	14 (5.6)	2.7～8.4	40 (15.9)	11.4～20.5	1 (0.4)	0～1.2	2 (0.8)	0.3～1.9
Total	343 (100)	38 (11.1)		18 (5.2)		40 (11.7)		1 (0.3)		2 (0.6)	

*PMD, personally managed rural dogs; SMD, systematically managed rural dogs.

Table 2. Prevalence of selected canine vector-borne disease pathogens in rural outdoor dogs according to sex

Category	Dogs examined *n* (%)	*D. immitis*		*M. haemocanis*		*B. gibsoni*		*A. phagocytophilum*		*H. canis*	
		No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)
SMD*											
Female	61 (66.3)	1 (1.6)	0～4.9	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
Male	31 (33.7)	1 (3.2)	0～9.5	4 (12.9)	0.9～24.9	0 (0)	-	0 (0)	-	0 (0)	-
PMD											
Female	161 (64.1)	19 (11.8)	0.4～5.8	5 (3.1)	0.4～5.8	19 (11.8)	6.8～16.8	0 (0)	-	2 (1.2)	0～3.0
Male	90 (35.9)	17 (18.9)	3.8～16.2	9 (10)	3.8～16.2	21 (23.3)	14.5～32.1	1 (1.1)	0～3.3	0 (0)	-
Total	343 (100)	38 (11.1)		18 (5.2)		40 (11.7)		1 (0.3)		2 (0.6)	

*PMD, personally managed rural dogs; SMD, systematically managed rural dogs.

found in the prevalence of *M. haemocanis* (*P*=0.651), *A. phagocytophilum* (*P*=0.544), or *H. canis* (*P*=0.394) infections between the different management systems. Simultaneous infection with more than one pathogen was not found in the SMD group. In contrast, in the PMD group, 29.1% (73/251) and 4.0% (10/251) of the dogs were infected with single and dual CVBD pathogens, respectively. Infection with three or more CVBD pathogens was not detected. Among the dogs simultaneously infected with CVBD pathogens (*n*=10), infection with *D. immitis/B. gibsoni* (*n*=5) was predominant, followed by infection with *D. immitis/M. haemocanis* (*n*=2), *B. gibsoni/M. haemocanis* (*n*=1), *B. gibsoni/A. phagocytophilum* (*n*=1), and *B. gibsoni/H. canis* (*n*=1).

Prevalence of CVBD pathogens by sex

The CVBD pathogen infection rates were analyzed according to sex, in female (*n*=161) and male (*n*=90) dogs in the PMD group. The proportion of overall CVBD pathogen infections in male dogs (*n*=41, 45.6%) was relatively higher than that in female dogs (*n*=42, 26.1%) (Table 2). The detection rate was highest for *D. immitis* (*n*=19, 11.8%) and *B. gibsoni* (*n*=19, 11.8%), followed by *M. haemocanis* (*n*=5, 3.1%) and *H. canis* (*n*=2, 1.2%) in female dogs, whereas *B. gibsoni* (*n*=21, 23.3%) infection was most common in male dogs followed by *D. immitis* (*n*=17, 18.9%), *M. haemocanis* (*n*=9, 10.0%), and *A. phagocytophilum* (*n*=1, 1.1%) infections. Significantly more male dogs were infected with *B. gibsoni* (*P*=0.017) and *M. haemocanis* (*P*=0.022). However, no significant difference was observed in the infection rates of *D. immitis* (*P*=0.124), *A. phagocytophilum* (*P*=0.180), or *H. canis* (*P*=0.288), based on the sex of the dogs.

Prevalence of CVBD pathogens by age

The results for the PMD group were analyzed according to age group, based on an age of <1 year (*n*=84),

Table 3. Prevalence of selected canine vector-borne disease pathogens in rural outdoor dogs according to age

Category	Dogs examined *n* (%)	*D. immitis*		*M. haemocanis*		*B. gibsoni*		*A. phagocytophilum*		*H. canis*	
		No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)	No. positive *n* (%)	95% CI (%)
SMD*											
<1 year	0 (0.0)	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
1 year	25 (27.2)	1 (4.0)	0～11.8	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
≥2 years	67 (72.8)	1 (1.5)	0～4.4	4 (6.0)	0.3～11.7	0 (0)	-	0 (0)	-	0 (0)	-
PMD											
<1 year	84 (33.5)	3 (3.6)	0.2～9.3	4 (4.8)	0.2～9.3	4 (4.8)	0.2～9.3	0 (0)	-	0 (0)	-
1 year	113 (45.0)	18 (15.9)	0.1～7.0	4 (3.5)	0.1～7.0	21 (18.6)	11.4～25.8	1 (0.9)	0～2.6	0 (0)	-
≥2 years	54 (21.5)	15 (27.8)	2.7～19.6	6 (11.1)	2.7～19.6	15 (27.8)	15.7～39.8	0 (0)	-	2 (3.7)	0～8.8
Total	343 (100)	38 (11.1)		18 (5.2)		40 (11.7)		1 (0.3)		2 (0.6)	

*PMD, personally managed rural dogs; SMD, systematically managed rural dogs.

1 year (*n*=113), and ≥2 years of age (*n*=54) (Table 3). In the <1-year-old group, pathogen infection was found in 11 dogs (13.1%), with the most prevalent pathogens being *B. gibsoni* (*n*=4, 4.8%) and *M. haemocanis* (*n*=4, 4.8%), followed by *D. immitis* (*n*=3, 3.6%). In the 1-year-old group, infection with at least one CVBD pathogen was detected in 40 dogs (35.4%), specifically with *B. gibsoni* (*n*=21, 18.6%), *D. immitis* (*n*=18, 15.9%), and *M. haemocanis* (*n*=4, 3.5%), in that order of prevalence. In the ≥2-year-old group (*n*=54), CVBD infection was found in 30 dogs (50.8%); the most prevalent pathogen was *D. immitis* (*n*=15, 27.8%) and *B. gibsoni* (*n*=15, 27.8%), followed by *M. haemocanis* (*n*=6, 11.1%), *H. canis* (*n*=2, 3.7%), and *A. phagocytophilum* (*n*=1, 0.9%). Significantly more dogs in the ≥2-year-old group were infected with *D. immitis* ($P<0.001$), *B. gibsoni* ($P=0.001$), and *H. canis* ($P=0.025$), whereas infection with *M. haemocanis* ($P=0.126$) and *A. phagocytophilum* ($P=0.542$) was independent of age.

DISCUSSION

The present study showed that the prevalence of *D. immitis* infection was much lower in the SMD group (2.2%) than in the PMD group (14.3%), although the dogs received heartworm treatment at least once a year during the study period. Previous studies have reported the prevalence of *D. immitis* infection in dogs as ranging from 9.9% to 50.3% in the ROK (Wee et al, 2001; Lim et al, 2010; Bell et al, 2012; Jung et al, 2012; Suh et al, 2017). As mosquitoes are distributed throughout the country, the opportunity for exposure was considered similar for all of the rural outdoor dogs (Lee et al, 2017). Nevertheless, our results showed differences in the prevalence of CVBD pathogen infections, and it may result from whether heartworm-preventive medications and chemoprophylaxis were administered strictly following the programmed schedule. According to Suh et al. (2017), 13.6% of the dogs that had received heartworm treatment were still seropositive for *D. immitis*. Notably, the survey conducted in the previous study revealed that the dog owners did not provide comprehensive heartworm treatment and only visited veterinarians for heartworm treatment an average of 2.02 times per year. Moreover, a survey of 503 sheltering agencies in US showed that only 57% of the agencies administered heartworm preventive drugs to shelter dogs, even though they have recognized guidelines for canine heartworm management (Colby et al, 2011). Based on the results of the present and previous studies, strict adherence to monthly treatment with the highly available and effective heartworm preventive drugs is important for successfully preventing *D. immitis* infection.

B. gibsoni infection showed a trend similar to that of *D. immitis* infection in our study, with no infection observed in the SMD group in contrast to that in the PMD group (15.9%). The infection rate in the PMD group was

much higher than that reported in military dogs (1.7%), indoor companion dogs (3.59%), and outdoor dogs (5.2%) in the ROK (Bell et al, 2012, Kim et al, 2017; Suh et al, 2017). Both the SMD and PMD groups were treated against tick once the parasite was observed. But the prevalence of *B. gibsoni* infection was dramatically different between the groups, which is attributed to environmental controlling efforts in managing the SMD group that included separation of each dog in room-like kennels, daily removal of vegetation, and cleaning of kennels. Rural housing dogs are often used as hunting dogs in the ROK; notably, hunting might increase tick infestation and blood contamination as a consequence of scratch or bite wounds from wild animals or other dogs infected with *Babesia* spp. Blood-to-blood contact through bite wounds might constitute an alternative major route for *B. gibsoni* transmission (Boozer and Macintire, 2005; Jefferies et al, 2007). However, Choe *et al* (2011) found that military working dogs receiving daily and periodic care from their owners and veterinarians, including ectoparasite preventive treatment, were free of ticks, despite their high levels of outdoor activities. Similar to the previous study, our result showed no infection of *B. gibsoni* in the SMD group. It suggests that control of surroundings is also important for preventing tick infestation and *B. gibsoni* transmission in dogs besides use of preventive medications.

The present study revealed the absence of *Candidatus Mycoplasma haematoparvum* and low infection rate for *M. haemocanis* in both the PMD and SMD groups. Different from our study, one previous study reported high prevalence of *Candidatus M. haematoparvum* and *M. haemocanis* infection in outdoor dogs (Suh et al, 2017). There is no information currently available regarding risk factors of *M. haemocanis* infection in dogs in the ROK. But Aquino et al. (2016) reported a significant correlation between the use of ectoparasite prophylaxis and a lower prevalence of infection, with a two-fold higher prevalence of hemoplasma infection in tick-infested dogs (21%) than in non-tick-infested dogs (11%). Thus, besides further study to identify the related risk factors, prevention of tick infestation is necessary for minimizing the risk of hemoplasma infection. On the other hand, *A. phagocytophilum* and *H. canis* infections were observed in only one and two dogs of the PMD group in the present study, respectively. However, these pathogens have been increasingly reported since they were first recorded in the ROK in 2016 (Lee et al, 2016; Kwon et al, 2017; Suh et al, 2017). Moreover, our results showed no significant association between the infection of *M. haemocanis*, *A. phagocytophilum*, and *H. canis* and either PCV or the presence of anemia, consistent with the results of previous studies (Barker et al, 2010; Novacco, 2010; Roura et al, 2010; Hetzel et al, 2012). Hence, hematological, biochemical, and other clinical findings of dogs infected with these pathogens would likely be misinterpreted as findings for healthy dogs when evaluating health status (Byun et al, 2007; Lim et al, 2010; Roura et al, 2010; Bell et al, 2012; Movilla et al, 2016; Novacco et al, 2016). Consequently, asymptomatic dogs infected with *M. haemocanis*, *A. phagocytophilum*, and *H. canis* might be left untreated and become reservoirs for these pathogens (Balakrishnan, 2016; Baneth and Cohn, 2016; Movilla 2016). Therefore, infections by these CVBD pathogens should be monitored continuously in dogs, regardless of their symptoms.

As evidenced by the overall low rate of CVBD pathogen infections in the SMD group, the application of preventive drugs strictly according to schedule, regular use of prophylaxis, and control of the breeding environment constituted important factors to prevent dogs from bites of arthropod vectors and infections by CVBD pathogens. Therefore, continuous education on the importance of strict adherence to scheduled application of preventive medications to dogs is necessary for veterinary practitioners and dog owners. Further, all the infected dogs in this study were clinically healthy, suggesting that CVBD pathogen infections are difficult to detect by general health check-ups. As the infected dogs left untreated might become competent reservoir hosts of the pathogens, periodic examinations and continuous monitoring are important for public health.

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REFERENCES

Aktas M and Ozubek SA. 2018. Molecular survey of hemoplasmas in domestic dogs from Turkey. Vet Microbiol 221: 94-97.

Aquino LC, Kamani J, Haruna AM, Paludo GR, Hicks CA, Helps CR, Tasker S. 2016. Analysis of risk factors and prevalence of haemoplasma infection in dogs. Vet Parasitol 221: 111-117.

Balakrishnan N, Musulin S, Varanat M, Bradley JM, Breitschwerdt EB. 2014. Serological and molecular prevalence of selected canine vector borne pathogens in blood donor candidates, clinically healthy volunteers, and stray dogs in North Carolina. Parasit Vectors 7: 116.

Baneth G and Cohn L. 2016. Canine hepatozoonosis. pp. 109-121. In: Day MJ(ed.). Arthropod-borne infectious diseases of the dog and cat. 2nd ed, CRC Press, Boca Raton, Florida, USA.

Barker EN, Tasker S, Day MJ, Warman SM, Woolley K, Birtles R, Georges KC, Ezeokoli CD, Newaj-Fyzul A, Campbell MD, Sparagano OA, Cleaveland S, Helps CR. 2010. Development and use of real-time PCR to detect and quantify *Mycoplasma haemocanis* and "*Candidatus Mycoplasma haematoparvum*" in dogs. Vet Microbiol 140: 167-170.

Bell DR, Berghaus RD, Patel S, Beavers S, Fernandez I, Sanchez S. 2012. Seroprevalence of tick-borne infections in military working dogs in the Republic of Korea. Vector Borne Zoonotic Dis 12: 1023-1030.

Birkenheuer AJ, Correa MT, Levy MG, Breitschwerdt EB. 2005. Geographic distribution of babesiosis among dogs in the United States and association with dog bites: 150 cases (2000-2003). J Am Vet Med Assoc 227: 942-947.

Boozer L and Macintire D. 2005. *Babesia gibsoni*: an emerging pathogen in dogs. Comp Cont Educ Pract 27: 33-42.

Byun JW, Park YI, Lee OS, Shim HS, Cho KM, Yoon SS. 2007. Prevalence of canine dirofilariasis of the stray dogs in Korea. Korean J Vet Public Health 31: 15-20.

Choe HC, Fudge M, Sames WJ, Robbins RG, Lee IY, Chevalier NA, Chilcoat CD, Lee SH. 2011. Tick surveillance of dogs in the Republic of Korea. Syst Appl Acarol 16: 215-222.

Colby KN, Levy JK, Dunn KF, Michaud RI. 2011. Diagnostic, treatment, and prevention protocols for canine heartworm infection in animal sheltering agencies. Vet Parasitol 176: 333-341.

Dantas-Torres F. 2008. Canine vector-borne diseases in Brazil. Parasit Vectors 1: 25.

Genchi C, Rinaldi L, Mortarino M, Genchi M, Cringoli G. 2009. Climate and *Dirofilaria* infection in Europe. Vet Parasitol 163: 286-292.

Hetzel NJ, Barker EN, Helps CR, Tasker S, Arteaga A, Barrs VR, Beatty J. 2012. Prevalence of canine haemotropic mycoplasma infections in Sydney, Australia. Vet Rec 171: 126.

Jefferies R, Ryan UM, Jardine J, Broughton DK, Robertson ID, Irwin PJ. 2007. Blood, bull terriers and babesiosis: further evidence for direct transmission of *Babesia gibsoni* in dogs. Aust Vet J 85: 459-463.

Jung BY, Gebeyehu EB, Seo MG, Byun JW, Kim HY, Kwak D. 2012. Prevalence of vector-borne diseases in shelter dogs in Korea. Vet Rec 171: 249.

Kim MJ, Chung TH, Na YH, Choi US. 2017. Seroprevalence of *Babesia gibsoni* in companion dogs in Korea by enzyme linked immunosorbent assay using recombinant BgTRAP antigen. J Vet Clin 34: 185-188.

Lee AC, Bowman DD, Lucio-Forster A, Beall MJ, Liotta JL, Dillon R. 2011. Evaluation of a new in-clinic method for the detection of canine heartworm antigen. Vet Parasitol 177: 387-391.

Lee AC, Montgomery SP, Theis JH, Blagburn BL, Eberhard ML. 2010. Public health issues concerning the widespread distribution of canine heartworm disease. Trends Parasitol 26: 168-173.

Lee DK. 2017. Ecological characteristics and current status of infectious disease vectors in South Korea. J Korean Med Assoc 60: 458-467.

Lee S, Lee SH, VanBik D, Kim NH, Kim KT, Goo YK, Rhee MH, Kwon OD, Kwak D. 2016. First molecular detection and phylogenetic analysis of *Anaplasma phagocytophilum* in shelter dogs in Seoul, Korea. Ticks Tick Borne Dis 7: 945-950.

Lim S, Irwin PJ, Lee S, Oh M, Ahn K, Myung B, Shin S. 2010. Comparison of selected canine vector-borne diseases between urban animal shelter and rural hunting dogs in Korea. Parasit Vectors 3: 32.

Movilla R, Garcia C, Siebert S, Roura X. 2016. Countrywide serological evaluation of canine prevalence for *Anaplasma* spp., *Borrelia burgdorferi* (sensu lato), *Dirofilaria immitis* and *Ehrlichia canis* in Mexico. Parasit Vectors 9: 421

Novacco M, Meli ML, Gentilini F, Marsilio F, Ceci C, Pennisi MG, Lombardo G, Lloret A, Santos L, Carrapiço T, Willi B, Wolf G, Lutz H, Hofmann-Lehmann R. 2010. Prevalence and geographical distribution of canine hemotropic mycoplasma infections in Mediterranean countries and analysis of risk factors for infection. Vet Micro-

biol 142: 276-284.

Otranto D, Dantas-Torres F, Breitschwerdt EB. 2009. Managing canine vector-borne diseases of zoonotic concern: part one. Trends Parasitol 25: 157-163.

Roura X, Peters IR, Altet L, Tabar MD, Barker EN, Planellas M, Helps CR, Francino O, Shaw SE, Tasker S. 2010. Prevalence of hemotropic mycoplasmas in healthy and unhealthy cats and dogs in Spain. J Vet Diagn Invest 22: 270-274.

Stull JW, Kasten JI, Evason MD, Sherding RG, Hoet AE, O'Quin J, Burkhard MJ, Weese JS. 2016. Risk reduction and management strategies to prevent transmission of infectious disease among dogs at dog shows, sporting events, and other canine group settings. J Am Vet Med Assoc 249: 612-627.

Suh GH, Ahn KS, Ahn JH, Kim HJ, Leutenegger C, Shin S. 2017. Serological and molecular prevalence of canine vector-borne diseases (CVBDs) in Korea. Parasit Vectors 10: 146.

Wee SH, Lee CG, Kim JT, 2001. Prevalence of *Dirofilaria immitis* infection in dogs of Chuncheon area. Korean J Vet Public Health 25: 229-232.