# Survey of *Ehrlichia canis* and *Borrelia burgdorferi* antibodies in dogs (German shepherd) reared in Korea

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Abstract: This study was conducted to survey of *Ehrlichia* (*E.*) canis and *Borrelia* (*B.*) burgdorferi antibodies among clinically healthy German shepherd dogs (116 females and 120 males) using a ELISA kit (SNAP 3Dx; IDEXX Laboratories, USA) in Korea. Whole blood samples are collected from the 236 dogs and are tested to detect *E. canis* and *B. burgdorferi* antibodies by using ELISA kit (SNAP 3Dx; IDEXX Laboratories, USA). Confidence interval comparisions revealed that dogs of 4-6 years have higher prevalence with a seropositive result (CI = 0.17 – 0.45) in *E. canis* than the other age groups but there are no differences among age groups in *B. burgdorferi*. Also, no differences with a seropositive result were found among different regions in *E. canis* and *B. burgdorferi* antibodies. In conclusion, this study was the first large scale survey of canine *E. canis* and *B. burgdorferi* antibodies in Korea and provide an useful reference for clinicians.

Key words: Borrelia burgdorferi, Ehrlichia canis, ELISA, survey, dog

#### Introduction

In recent years, many epidemiological surveys of *Ehrlichia* (*E.*) canis and *Borrelia* (*B.*) burgdorferi have been performed in many countries [3, 7, 8, 12] and most of the animals tested were ticks, cats and dogs. In Korea, molecular epidemiological study for *Ehrlichia* and *Anaplasma* spp. in ticks [2] and the serological diagnosis of Lyme disease in ticks [6, 9] were recently reported, but there were no reports on the seroprevalence of dogs either as carriers or patients. This study was performed to survey of *E. canis* and *B. burgdorferi* antibodies among clinically healthy dogs using a ELISA kit (SNAP 3Dx; IDEXX Laboratories, USA) in Korea.

#### Materials and Methods

## Surveyed areas and animals

The present survey was carried out in the province of Kangwon, mountain area, located in the eastern of Korea, Kyunggi, urban area, located in the western of Korea, Chungnam, agricultural area, located in the mid-western of Korea and Kyungnam, agricultural area, located in the southern of Korea. The 236 dogs (116 females and 120 males) investigated were all of the German shepherd breed from April 2005 to September 2005. Most were used as guard dogs but some were used in hunting. All had been reared in Korea. The sample population was randomly chosen to enter the veterinary clinics for Dirofilaria immitis examination and/or annual vaccination. However, all dogs were not conducted by vaccination or treatment for B. burgdorferi and for E. canis. Their age range was from less two years to over six years (mean 6.7 years old). Blood was collected from the cephalic vein of the dogs and then was stored in a freezer at -20°C for the analysis.

#### **ELISA** test

Blood samples were examined for antibodies to *E. canis* and *B. burgdorferi* using a commercial ELISA kit (SNAP 3Dx; IDEXX Laboratories, USA). The sensitivity and specificity were found to be 0.71 and

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Table 1. Logistic regression analysis to detect factor associated to antibody response to E. canis and B.burgdoriferi in dogs

	E. canis					B. burgdoriferi				
Term	No. of examined	No. of positives	Prevalence	True prevalence	CI <sub>95%</sub>	No. of examined	No. of positives	Prevalence	True prevalence	CI <sub>95%</sub>
Gender										
Females <sup>a</sup>	115	15	13	18.3	0.09-0.28	115	3	2.6	3.7	0.01-0.08
Males	121	14	11.6	16.3	0.08-0.25	121	7	5.8	8.2	0.02-0.14
Age(yr)										
< 4ª	69	2	2.9	4.1	0.02-0.10	69	2	2.9	4.1	0.02-0.10
4~6	78	17	21.8	30.7	0.17-0.45	78	3	3.8	5.4	0.01-0.11
> 6	89	10	11.2	15.8	0.06-0.26	89	5	5.6	7.9	0.01-0.15
Provinces		'							,	
Kyunggia	53	3	5.7	8	0.01-0.17	53	3	5.7	8	0.01-0.17
Kangwon	79	9	11.4	16.1	0.06-0.26	79	3	3.8	5.4	0.01-0.11
Chungnam	36	6	16.7	23.5	0.06-0.41	36	1	2.8	3.9	0.04-0.12
Kyungnam	68	11	16.2	22.8	0.10-0.36	68	3	4.4	6.2	0.01-0.13

CI<sub>95%</sub>: confidence intervals (95%). <sup>a</sup>Variable used as a reference value.

1.00 obtained based on the sample size 21 and 46, respectively [4]. All procedures was performed according to the manufacturer's recommendations.

# True prevalence

The true prevalence based on the proportion of test-positive animals was estimated with the Rogan-Gladen-formula [11].

$$P_{RG} = \frac{A_P + S_P - 1}{S_P + S_P - 1}$$

Where  $A_P$  is the proportion of test-positive animals and  $S_E$  is the sensitivity and  $S_P$  is the specificity.

## Statistical analysis

Statistical analysis was carried out with comparisions of 95% confidence intervals (of the true prevalence). The prevalences were compared, among age-groups (<4 years, 4-6 years and >6 years), genders and provinces of residence, version 10.0 of the SPSS for Windows software package (SPSS, USA).

## Results and discussion

In our study, the seroprevalence of E. canis in dogs were 18.3% in female and 16.3% in male dogs in E. canis, there was no significant difference between the two groups, which is in agreement with the report by Botros *et al.* [1]. Our results showed that 4.1% in < 4

year old group, 30.7% in 4-6 year old group and 15.8% in older than 6 year old group. The comparisions of confidence intervals revealed that the prevalence of 4-6 year dogs is statistically significantly higher then that of dogs of less then 4 years. The results is similar to those of Rodriguez-Vivas et al. [10]. The results showed no significant differences of seroprevalence among mountain, agricultural and urban areas. Possible explanations include the exposure to the vector ticks in these areas. The present study revealed that no symptoms were observed in seropositive dogs. It may be latent infection or cross reactions that occur between the antigens of E. canis and Ehrlichia spp. In our study, the seropositive rate was 3.7% in male and 8.2% in female dogs, which was no significant difference between the two groups. Joppert et al. [5] also reported that seropositive rate of B. burgdorferi was not differences between different gender could be found. Our results showed 4.1% in <4 year old group, 5.4% in 4-6 year old group and 7.9% in older than 6 year old group. Since all the confidence intervals were shown to be overlapped with others there were no differences in prevalence between genders, ages, prairess. The present study also revealed that no symptom was observed in seropositive dogs. There may be latent infection or cross reactions between the antigens of B. burgdorferi and other related bacteria. The present study showed no significant differences of B. burgdorferi was observed among different regional

distribution.

In conclusion, these results provide an useful reference for clinicians. Further genomic studies is needed to confirm the zoonotic potential of *E. canis* and *B. burgdorferi* isolated from dogs in Korea.

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