

## Prevalence state of canine brucellosis in South Korea during 2015 and 2016

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**Abstract:** We investigated the prevalence of canine brucellosis in companion and stray dogs between March 2015 and December 2016 and determined the disease characteristics based on the geographic distribution, sex and age of the dogs in South Korea. We conducted a large-scale survey using serological and bacteriological tests. Samples were collected from 2,394 dogs (1,825 companions and 569 strays). Thirty (1.3%) samples were positive for *Brucella canis* antibodies including 16 (0.9%) from companion dogs and 14 (2.5%) from stray dogs. Two (1.0%) of the 196 samples cultured from the stray dogs were positive. When compared with male dogs, the female companion and stray dogs had a significantly higher prevalence of brucellosis. Moreover, the prevalence of canine brucellosis was significantly higher in stray dogs older than 6 years and the prevalence of the disease in companion dogs was highest in Incheon (2.1%) and Jeolla (2.1%) provinces. Stray dogs from the Daejeon metropolitan area had the highest prevalence of brucellosis (7.9%). National control measures for canine brucellosis have not previously been implemented. Our findings suggest that appropriate screening tests and control measures are necessary to improve the health of dogs and to protect public health in Korea, particularly with the rapid growth of the companion animal industry.

**Keywords:** *Brucella canis*, bacteriology, brucellosis, prevalence, serology

### Introduction

Brucellosis is an important zoonotic disease that poses serious public health risks and is associated with economic losses in worldwide [10]. The *Brucella* (*B.*) genus is composed of six “classical” species based on host preference and genetic analysis: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* [11]. Although dogs can be infected by *B. abortus*, *B. melitensis*, and *B. suis*, the infection of dogs with *B. canis* is the most common, and results in serious clinical events including spontaneous abortion [25].

The infection can be transmitted between dogs by venereal, oronasal, or conjunctival routes [25]. After *Brucella* enters the body, it is phagocytized by macrophages and travels to the lymph nodes and target reproductive (steroid-sensitive) organs. The bacteria spread via the bloodstream to other tissues such as intervertebral discs, kidneys, and eyes. Bacteremia is evident 1–4 weeks after infection and can last for several years [11].

Human brucellosis is one of the most important world-

wide zoonotic diseases and is re-emerging in some countries, especially Mediterranean areas including Syria, Iraq, Turkey, Iran, and Saudi Arabia. Human brucellosis is caused by the infection of *B. abortus*, *B. suis*, *B. melitensis*, and *B. canis* [17]. Although there were no reported human cases of *B. canis* infection in Korea, more attention is warranted for this disease due to the increasing ownership of companion dogs.

Carmichael [7] firstly identified *B. canis* in 1966 as the cause of abortion among beagles in the USA. Since then, the disease has been reported in several countries including Argentina [21], Canada [4], Germany [26], Mexico [9], Nigeria [1, 6], and Zimbabwe [8]. In Asia, the disease has been reported in China [13], Japan [20], and Malaysia [14].

In Korea, *B. canis* was firstly isolated in outdoor dogs in 1984 [18]. After that, several studies for the seroprevalence of canine brucellosis have been conducted [3, 19, 22], especially in breeding kennels. However, there was no available information regarding the distribution and/or prevalence of canine brucellosis in companion and stray dogs. Thus, the aim of the present study was to investigate the prevalence of

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canine brucellosis in companion and stray dogs in Korea, and to determine the disease characteristics based on the geographic distribution and the sex and age of dogs.

## Materials and Methods

### Animals

Whole blood or serum samples of 2,394 dogs from 2 months to 23 years old (1,160 females and 1,234 males) were used in this study. We classified the dogs into 2 groups; companion dogs and stray dogs, based on their living conditions. Between March 2015 and December 2016, blood or serum samples of companion dogs ( $n = 1,825$ ) from 17 animal hospitals and stray dogs ( $n = 569$ ) from 5 dog shelters were collected (Table 1). The overall sample size was determined using a random sampling technique [23]:

$$n = \frac{1.96^2 p_{exp} (1 - p_{exp})}{d^2},$$

where  $n$  = required sample size,  $p_{exp}$  = expected prevalence, and  $d$  = desired absolute precision. The expected prevalence, using a 2-mercaptoethanol rapid slide agglutination test (2-ME RSAT) in the companion and stray dogs was considered as 1.5% and 8.2%, respectively [3], with a desired absolute precision of 5%. Thus, the required sample size was 23 companion dogs and 115 stray dogs. For statistical analysis, the sex and age of the dogs, and their geographic regions (Gyeonggi, Gangwon, Chungcheong, Gyeongsang, Jeolla province, Seoul special city, Incheon, Daejeon, Gwangju, Ulsan metropolitan city, and Jeju special self-governing province) were recorded.

### Sample preparation

Approximately 2–3 mL of whole blood was collected from the stray dogs using heparin as an anticoagulant for the serological test and bacterial isolation. For the serological test, blood was centrifuged at 4°C, at  $1,579 \times g$  for 30 min. The plasma was collected and inactivated at 56°C for 30 min, then stored at –20°C until used.

Because only serum samples were obtained from the animal hospitals, bacterial isolation could not be performed in companion dogs. The serum samples were stored at –20°C until needed.

### Immunochromatographic test

Serum or plasma samples were analyzed using an immunochromatographic test (ICT) for the serological test of canine brucellosis (BioNote, Korea), according to the manufacturer's instructions. Serum from dogs previously naturally infected with *B. canis* was used as the positive control.

### Blood culture and identification

After separating plasma from whole blood, the buffy coat or blood clot was used for bacterial isolation. The buffy coat layer or blood clot were inoculated in tryptose phosphate broth (BD Bioscience, USA) containing 5% fetal bovine serum (Gibco, USA) and incubated at 37°C under aerobic conditions for 30 days. Every 7 days the broth cultures were inoculated on sheep blood agar and incubated for 3–4 days at 37°C. Suspected colonies were selected and purified by further cultivation on sheep blood agar for 3–4 days at 37°C. Because it was impossible to successfully isolate the bacteria from the small amount of blood, the numbers of tested samples for serology were not matched to those for bacterial isolation.

For the identification of the bacteria, genomic DNA of the isolates was extracted using a QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's protocol. The identification of the isolates was confirmed using the novel Bruce-ladder multiplex polymerase chain reaction (PCR) assay [15].

### Statistical analysis

A Chi-square test was used to evaluate the association between the seroprevalence of *Brucella* and living condition, sex, age of the dogs, and the geographic region. All statistical analyses were performed using SPSS software (ver. 21.0; IBM, USA). A  $p$  value less than 0.05 was considered as statistically significant.

## Results

The detailed results of serology, bacterial culture, and overall prevalence of canine brucellosis are shown in Table 2.

In this study, canine brucellosis was diagnosed with ICT and bacterial culture. *Brucella* infection was determined if one or both tests were positive. Out of 2,394 samples, 30 (1.3%) dogs were positive for *B. canis* antibodies. Among

**Table 1.** Number of dogs examined for the prevalence of *Brucella canis*

Age (yr)	Companion dogs		Stray dogs	
	Male ( $n$ )	Female ( $n$ )	Male ( $n$ )	Female ( $n$ )
< 2	243	173	102	54
2–6	225	232	161	96
≥ 6	439	513	64	92
Subtotal	907	918	327	242
Total	1,825		569	

**Table 2.** Results of ICT and bacterial culture for *Brucella canis* between companion and stray dogs

Group	ICT		Bacterial culture		ICT or bacterial culture	
	Number of tested	Number of positive (%)	Number of tested	Number of positive (%)	Number of tested	Number of positive (%)
Companion dogs	1,825	16 (0.9)	NT	–	1,825	16 (0.9)
Stray dogs	569	14 (2.5)	196*	2 <sup>†</sup> (1.0)	569	14 (2.5)
Total	2,394	30 (1.3)	196	2 (1.0)	2,394	30 (1.3)

\*Bacterial culture was performed in 196 out of 569, due to the restricted volume of blood samples. <sup>†</sup> ICT also positive. ICT, immunochromatographic test; NT, not tested.

**Table 3.** Prevalence of *Brucella canis* infection in dogs according to the sex, age, and geographic region in Korea

Group		Companion dogs			Stray dogs		
		Number of dogs tested	Number of positive results (%)	<i>p</i> value	Number of dogs tested	Number of positive results (%)	<i>p</i> value
Sex	Male	907	3 (0.3)	0.013	327	3 (0.9)	< 0.001
	Female	918	13 (1.4)		242	11 (4.5)	
Age (yr)	< 2	416	2 (0.5)	0.192	156	2 (1.3)	0.007
	2–6	457	7 (1.5)		257	3 (1.2)	
	≥ 6	952	7 (0.7)		156	9 (5.8)	
Geographic region	Gyeonggi	184	2 (1.1)	0.541	180	1 (0.6)	< 0.001
	Gangwon	383	4 (1.0)		NT	–	
	Chungcheong	148	1 (0.7)		NT	–	
	Gyeongsang	54	1 (1.9)		NT	–	
	Jeolla	97	2 (2.1)		133	0 (0.0)	
	Seoul	209	2 (1.0)		NT	–	
	Incheon	142	3 (2.1)		NT	–	
	Daejeon	148	0 (0.0)		126	10 (7.9)	
	Gwangju	99	0 (0.0)		130	3 (2.3)	
	Ulsan	231	1 (0.4)		NT	–	
	Jeju	130	0 (0.0)		NT	–	

these, 16 (0.9%) were from companion dogs and 14 (2.5%) were from stray dogs. Blood culture could be performed only for 196 stray dogs; *B. canis* was isolated from 2 dogs (1.0%) and was clearly confirmed by the PCR method. These dogs were also positive for *B. canis* antibodies using ICT.

The status of *Brucella* infection associated with the sex and the age of dogs, and the geographic regions is shown in Table 3. In companion dogs, female dogs had significantly higher positivity (1.4%) to *B. canis* than the males (0.3%,  $p < 0.05$ ). Moreover, the prevalence of *B. canis* in female stray dogs (4.5%) was also significantly higher than in males (0.9%,  $p < 0.05$ ).

According to the age of the dogs, the prevalence of *B. canis* in dogs under 2 years, 2–6 years, and over 6 years old was recorded as 0.5% (2/416), 1.5% (7/457), and 0.7% (7/952), respectively ( $p > 0.05$ ) in companion dogs. The prevalence in stray dogs under 2 years, 2–6 years, and over 6 years old was 1.3% (2/156), 1.2% (3/257), and 5.8% (9/156),

respectively ( $p < 0.05$ ). Hence, *B. canis* infection is more common in stray dogs that are over 6 years in Korea.

The results were analyzed according to 11 different areas for the companion dogs and 4 different areas for the stray dogs. According to the geographic region, the higher prevalence for the companion dogs was recorded in Incheon metropolitan city and Jeolla province with a rate of 2.1%. However, the prevalence for stray dogs was highest in Daejeon metropolitan city with a rate of 7.9%. The results for the prevalence of canine brucellosis based on the geographic regions was revealed as statistically significant different in stray dogs ( $p < 0.05$ ), but not in companion dogs.

## Discussion

The diagnosis of canine brucellosis can be made by clinical laboratory findings, semen examination, serological test, bacterial isolation, and genetic detection of isolates [11]. The

serological tests include the 2-ME RSAT, tube agglutination test, indirect fluorescent antibody, cell wall agar gel immunodiffusion, cytoplasmic agar gel immunodiffusion, enzyme-linked immunosorbent assay, and ICT [12]. These serologic tests show variable sensitivity and specificity. The ICT is simple to perform and could be potentially used in routine clinical practice [25]. The ICT kit is broadly used as a national standard method in all diagnostic laboratories for the canine brucellosis in Korea. Therefore, we used ICT for the sero-diagnosis of canine brucellosis to clarify the prevalence of *B. canis* in companion and stray dogs in this study.

Although several reports regarding the occurrence of canine brucellosis have been conducted, mainly in breeding kennel dogs [18, 19, 22], there is very limited information for canine brucellosis in Korea. In addition, the prevalence of canine brucellosis may vary according to the test method and living condition of the target animals. In a previous study of 501 dogs in Korea, the seroprevalence rates of *B. canis* in 69 indoor dogs, 177 kennel dogs, and 225 stray dogs were 1.5%, 17.5%, and 8.2%, respectively, using 2-ME RSAT [3]. However, the sero-positive rate of *B. canis* in breeding kennel dogs was 14.1% using ICT, whereas 0% was recorded in companion dogs and stray dogs [3]. In the present study, we surveyed the prevalence of canine brucellosis on a large scale using 2,394 samples from companion dogs and stray dogs. The overall sero-positivity in companion and stray dogs was higher than reported in the previous study, based on the results of ICT in this study. In addition, the positive rate for *B. canis* in stray dogs (2.5%) was about three times higher than in companion dogs (0.9%). This result of a diagnostic tendency based on the living conditions of dogs is similar to a previous study [5], and may be closely related to the influence of uncontrolled mating and/or inadequate veterinary care in stray dogs.

In this study, female companion and stray dogs showed a significantly higher prevalence of brucellosis than the males. The different sexual prevalence in this study is very similar to previous studies in other countries [6, 8]. Infected female dogs shed *B. canis* during estrus and following abortion, and other dogs are infected through oronasal contact with vaginal discharge. Infected males can also transmit *B. canis* via the venereal route to females through the seminal fluid and urine. Seminal fluid and urine have been implicated as sources of infection from males that harbor organisms in their prostate gland and epididymis [11]. If a single male dog is infected with *B. canis* and then mates with several females, bacteria can be transmitted to other female dogs through the infected semen [6].

*Brucella* infection in dogs is reported to be age-dependent, due to the longer period of exposure in adult dogs [2]. In domestic dogs, sexual maturity occurs between the age of 6 to 12 months for both males and females, although this can be delayed until up to two years of age for some large breeds. In this study, older stray dogs ( $\geq 6$  years) had a higher prevalence (5.8%) of brucellosis than younger ones (1.2–

1.3%). Therefore, older dogs may play an important role in the transmission of *B. canis* to stray dogs in Korea. However, the prevalence of this disease in companion dogs is not age-dependent.

There has been some information on the prevalence of canine brucellosis at the restricted region in Korea. In this study, the seropositive rates of companion dogs in each region were ranged from 0.0% to 2.1%. Collected samples from Daejeon, Gwangju metropolitan city, and Jeju special self-governing province showed all negative result. However, in stray dogs, the highest rate of canine brucellosis was obtained in Daejeon metropolitan city. Although the reason of great serologic difference between companion dogs and stray dogs in same region is still unknown, stray dogs should be strictly controlled because some of them with carrier stage can transmit the bacteria to other dogs by several routes. Although limited information was available in this study because of the samples for stray dogs in restricted areas, more in-depth and continuous nationwide survey is necessary to monitor the precise prevalence of canine brucellosis in Korea.

For the screening of canine brucellosis, several serological methods to detect antibody are used in many countries. However, definitive diagnosis of *Brucella* infection depends on the antigen detection methods such as blood culture and nucleic acid amplification using PCR [16]. Bacteremia starts between 2 and 4 weeks post-infection and persists for about 6 months, becoming intermittent over at least one year [11]. We tried to isolate the *B. canis* organism from whole blood samples of dogs in this study. Unfortunately, only serum samples of companion dogs without blood were obtained from the local animal hospitals. Hence, we were could not conduct bacterial culture for companion dogs. Moreover, a follow-up study against seropositive companion dogs could not be performed because of the difficulty in continuous sampling. In addition, bacterial culture was performed on a limited number of stray dogs due to the lack of blood volume. The overall antigen positive rate using bacterial isolation was lower than the antibody positive rate through ICT in stray dogs. A lower antigen positive rate might be associated with a higher chronic infection stage in stray dogs or with artificial damage to the small volume of blood during sample processing. Moreover, bacteria cannot be cultured if the animal has received antibiotic treatment previously [24]. In addition, bacteremia of *B. canis* can be intermittent, and there is possibility of low number of organisms in the one point blood sample. To increase the isolation rate, repeated culture for consecutive blood sample may be necessary.

Bovine brucellosis, caused by *B. abortus*, is one of the most common zoonoses in Korea. The enforcement of control measures such as test and slaughter have led to a great reduction of the incidence of bovine brucellosis. However, there is currently no national control program for canine brucellosis in Korea. Although the prevalence of canine brucellosis is extremely low, zoonotic *B. canis* infections circulating

in companion and stray dogs can affect animal owners in Korea. Therefore, more stringent screening tests and effective control measures are warranted in Korea, particularly with regards to public health and the increase in popularity of companion animals.

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