

# Investigation of *Brucella canis* infection in public animal shelters and kennels in Incheon

Ji-Young Hong\*, Jin-Su Park, Tae-Ho Han, Hyun-Soon Hwang

Incheon Metropolitan City Institute of Health & Environment

(Received 3 December 2009, accepted in revised from 26 February 2010)

## Abstract

This survey was performed to investigate the seroprevalence of antibodies to *Brucella canis* in dogs from public animal shelters and breeding kennels in Incheon. A total of 402 dogs selected randomly were tested serologically by using immunochromatographic antibody test kit. None of 289 dogs in public animal shelters were sero-positive and 10 (8.9%) of 113 dogs in breeding kennels were sero-positive. 5 (4.4%) strains of *Brucella canis* were isolated from 10 sero-positive dogs' blood. Antimicrobial susceptibility test was carried out by Disk diffusion method. They were susceptible to tetracyclines, quinolones, aminoglycosides and combination amoxicillin with clavulanic acid.

**Key words** : *Brucella canis*, Public animal shelter, Kennel, Antibody, Susceptibility

## INTRODUCTION

*Brucella canis* (*B. canis*) was detected from a abortus for the first time when Chamichael and Kenny investigated the causes of abortion at the beagle-breeding farm in America in 1966. Then it was called *B. canis* after biochemical characterization by Brunner in 1968 (Carmichael and Kenney, 1968a, 1968b; Kim et al, 2007).

As an aerobic gram-negative,  $0.5 \sim 0.7 \times 0.6 \sim 1.5 \mu\text{m}$  wide, nonmotile rod, *B. canis* forms the translucent and rosy micro-colonies which are 1~1.5mm in diameter after cultured for 3~5 days. *B. canis* has been reported from all over the world, for example, Mexico, Argentina, Europe and Japan etc, since detected in America for the first time. *B. canis* has also been reported in the guinea pig, mice, rabbit. In addition, cat has been infected under experiment condition (Kim et al, 2006).

*B. canis* infection in human is not as serious as in dogs, but brucellosis is regarded as zoonosis because human can be naturally infected by direct contact with the infected dog and laboratory exposure (Park and Oh,

2001; Kim et al, 2006; Carmichael and Kenney, 1968a).

Canine brucellosis has no symptom in general, but it causes the abortion without prodrome in female, which is between 45 and 60 days of pregnancy. Furthermore, canine brucellosis causes the prostatitis and atrophy of testis in male. Especially, there are lots of inflammatory cells and abnormal sperm cells 3 months after infected.

Infertility can occur in chronic disease because of azoospermia and aspermato-genesis (Kim et al, 2006; Kim et al, 2007).

Canine brucellosis spreads by close contact or intake from the abortus, afterbirth and leukorrhea. And the bacilli is discharged at regular intervals through semen and uria of the infected dogs.

The dogs with *B. canis* need to be slaughtered in a bid to prevent the bacilli spreading as canine brucellosis is legal communicable disease II, but the therapeutics is also selected because they are thought to be companion of human and the effect of treatment by antimicrobial is being reported recently.

In this study, prevalence of canine brucellosis and antimicrobial susceptibility of the isolated bacilli from infected dogs were determined in public animal shelters

\* Corresponding author: Ji-Young Hong, Tel. +82-32-440-5650, Fax. +82-32-440-8863, E-mail. hjy3018@korea.kr

and kennels in Incheon.

## MATERIALS AND METHODS

### Posted materials

This study was conducted for a year from January to December in 2008 and we investigated for a total of 402 dogs in 6 public animal shelters and in 5 breeding kennels which were selected randomly. For serological test, about 0.1ml of whole blood was collected from cephalic vein of each dog. And then about 3ml of blood was collected from jugular vein of the antibody-positive dogs to isolate the causative bacilli before they were transferred to the tube (Becton Dickinson) containing sodium heparin.

### Serologic method

Antibody tests were performed by using C. brucella Ab test kit (Bionote co., Korea) which is based on immuno-chromatography. And we read the result 25~30 minutes after a 1 drop of the whole blood and 4~5 drops of diluent.

### Isolation and identification of *B. canis*

To isolate *B. canis*, shaking culture was done at 37°C after inoculated into 5ml of tryptic soy broth (Difco, TSB) and 1.0~1.5ml of clotted blood in a lower layer which was from the centrifugation of 3ml whole blood collected from antibody-positive dogs. And we detected

the presence of colonies after taking 1 loop every third day during cultured in broth for 30 days and then smearing on tryptic soy agar (Difco, TSA). In addition, *B. canis* was identified by confirming colonization, gram-staining and by doing a biochemical test.

### Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed according to Disk Diffusion method of CLSI (Clinical and Laboratory Standard Institute) (Kim et al, 2007; CLSI 2007).

After strains confirmed as *B. canis* were inoculated to 5ml of TSB and incubated them for 24hrs at 37°C, it was diluted by 1 : 100. Antimicrobial susceptibility test was carried out to using the dilutions with 15 antimicrobials: minocycline, doxycycline, tetracycline, ciprofloxacin, norfloxacin, sulfa -methoxazole trimethoprim, amoxicillin / clavulanic acid, ampicillin, kanamycin, gentamycin, streptomycin, amikacin, rifampicin, cephalothin, cephalazolin.

## RESULTS

As a result of antibody test, the overall antibody-positive rate in dogs of public animal shelters (289 dogs) and breeding kennels (113dogs) was 2.5% (10 dogs) among a total of 402 dogs.

In the comparison of antibody-positive rate for *B. canis* between different breeding spot, none of dogs was positive in the case of public animal shelters, while 10

**Table 1.** Antibody-positive rate of *B. canis* in public animal shelters and breeding kennels

Breeding spot	No. of test	No. of positive	Detective rate (%)
Public animal shelters	289	0	0.0
Breeding kennels	113	10	8.8
Total	402	10	2.5

**Table 2.** Comparison of antibody-positive rate for *Brucella canis* by sex

Sex	Public animal shelters and breeding kennels			Breeding kennels		
	No. of test	No. of positive dogs	Detective rate (%)	No. of test	No. of positive dogs	Detective rate (%)
Male	167	2	1.2	24	2	8.3
Female	235	8	3.4	89	8	9.0
Total	402	10	2.5	113	10	8.8

of 113 dogs (8.8%) were positive in the case of breeding kennels (Table 1).

In the comparison of antibody-positive rate for *B. canis* by sex, 2 cases among 167 dogs (1.2%) were positive in the case of male, while 8 of 235 dogs (3.4%) were positive in the case of female, so the rates were similar each other. In breeding kennels where antibody-positive dogs were confirmed, the comparison of the antibody-positive rate by sex were also similar each other (Table 2).

*B. canis* was isolated in 5 cases among 10 dogs which was antibody-positive. The overall detective rate was 50.0%, and the rate in antibody-positive female as *B. canis* was isolated only from all the female (Table 3). And all 5 dogs in which *B. canis* was isolated were female which had experience in mating and parturition.

Also, susceptibility for antimicrobials was reported that Tetracyclines was most susceptible, followed by Quinolones, Amino-glycosides (Table 4).

**Table 3.** Isolation of *Brucella canis* from antibody-positive dogs

Sex	No. of sero-positive	Isolation	
		No. of dogs with <i>B. canis</i>	Detective rate (%)
Male	2	0	0.0
Female	8	5	62.5
Total	10	5	50.0

**Table 4.** Result of antimicrobial susceptibility test

Antimicrobials (Concentration/Disc)	Susceptibility (n <sup>†</sup> )		
	Resistant	Intermediate	Susceptible
Minocycline (30µg)	–	–	5
Doxycycline (30µg)	–	–	5
Tetracycline (30µg)	–	–	5
Ciprofloxacin (5µg)	–	1	4
Norfloxacin (10µg)	–	–	5
Sulfamethoxazole trimethoprim (25µg)	5	–	–
Amoxicillin&clavulanic acid (30µg)	–	–	5
Ampicillin (10µg)	1	1	3
Kanamycin (30µg)	1	1	3
Gentamycin (10µg)	1	1	3
Streptomycin (10µg)	–	1	4
Amikacin (30µg)	–	3	2
Rifampin (5µg)	–	1	4
Cephalothin (30µg)	5	–	–
Cefazolin (30µg)	4	1	–

<sup>†</sup>No. of the strain

## DISCUSSION

Brucellosis is an important zoonotic bacterial disease caused by different species of genus *Brucella*, that are pathogenic for a variety of animals and human beings worldwide. Canine brucellosis caused by *B. canis* has a considerable impact on human health, because dog is the most popular companion animal. So, the dogs with *B. canis* need to be slaughtered in a bid to prevent the bacilli spreading, but the therapeutics is also selected because the effect of treatment by antimicrobial is being reported recently and dogs are thought to be companion of human.

We investigated the actual prevalence of *B. canis* in dogs from public animal shelters and breeding kennels in the Incheon area, and isolated *B. canis* from antibody-positive dogs and carried out a antimicrobial susceptibility test by isolated bacilli.

From January to December in 2008, serum samples collected from a total of 402 dogs were examined, 10 dogs (2.5%) were antibody-positive and all of the antibody-positive dogs were breeding dogs in kennels. Antibody-positive ratio of the five breeding kennels was 8.8%. and we isolated *B. canis* from 5 samples among the 10 antibody-positive samples.

None of dogs was antibody-positive even if the number of dogs surveyed from public animal shelters was larger than that from kennels.

It is thought that most of abandoned dogs were also raised as a companion of human before abandoned so they didn't have lots of chances to contact with the infected dogs and most of them had few experience for breeding or parturition because they were 1~2 years old when captured. And also Moon et al (1999b) reported higher prevalence of brucellosis in prolific dogs over 3 years old.

On the other hands, Koh et al (2007) reported that all the cases were *Brucella canis*-negative in abandoned dogs which were selected randomly in public animal shelters of Kwang-ju metropolitan city. They thought that was because the size of surveys was small. Stemming from this, it is thought that the survey of all the dogs for ages, experiences of parturition or sterilization is more desirable than selected randomly in a bid to detect the brucellosis.

Also, the serological detective rate in 113 dogs of 5 breeding kennels was 8.8% (10 cases) and it is similar to a previous report by Kim and Lee (2003a) in which 12 of 134 dogs (9.0%) were antibody-positive in 7 breeding kennels. In contrast, much different results were reported from surveys conducted on breeding kennels which experienced abortion and reproduction disorders 63 of 126 dogs (50.0%) by Kim and Lee (2003a) 143 of 267 dogs (53.6%) by Kim et al (2007) and 81 of 195 dogs (41.5%) by Park and Oh (2001).

In the present study, *B. canis* was successfully isolated from 5 of 10 serologically positive dogs (50.0%) Similarly, 37 of 75 (49.3%) were positive in the report by Kim and Lee (2003a), 81 of 195 (41.5%) by Park and Oh (2001), and 20 of 33 (60.6%) by Moon et al (1999b), indicating that about a half of *B. canis* was isolated in antibody-positive dogs. On the other hands, 1 bacilli was isolated in antibody-negative dogs by Kim et al (2007) and Park and Oh (2001). And Kim and Lee (2003a) reported that *B. canis* was isolated in 15 among 185 antibody-negative dogs (8.1%), Moon et al (1999b) reported 1 more antibody-positive dog as a result of the second test for antibody-negative dog. Considering these, it is thought that the serological tests and identification of the causative bacilli for all the dogs in addition to regular retests need to be done in a bid to exterminate

canine brucellosis.

In the comparison of antibody-positive rate for *B. canis* in the breeding kennels by sex, 2 cases among 24 dogs (8.3%) were positive in the case of male, while 8 of 89 dogs (9.0%) were positive in the case of female, so the rates were similar each other. Also, on survey in the abortion occurred breeding kennels by Moon et al (1999b), 3 cases among 6 dogs (50.0%) were positive in the case of male, while 30 of 56 dogs (53.5%) were positive in the case of female. It is thought that the difference in dogs surveyed results in a gap between both antibody-positive rates.

Tetracyclines was the most susceptible, followed by Quinolones, Rifampins, Aminoglycosides in the antimicrobial susceptibility test. That was similar to a previous study conducted on different strains isolated in Korea (Kim and Lee, 2003b).

Kim et al (2006) reported the efficacy of antibiotic therapy with streptomycin, penicillin, enrofloxacin and doxycycline. Moon et al (1999a) reported that 28 infected dogs had become abacteremic and serologic titers were declined after the first antibiotic treatment and abortion due to *B. canis* infection could be prevented by antibiotic therapy.

*B. canis* often incubates in the prostate or lymph node because it is an intracellular bacilli. It is difficult to eradicate the causative bacilli by antibiotic therapy for a short period, so slaughter is recommended to eradicate the disease (Antonio and Martin, 1995). Nevertheless, a lot of dog-lovers choose treatment rather than euthanasia for companion dogs. For companion animals, we do think it is effective to quarantine the infected dogs at first in a bid to prevent human infection, and to carry out sterilization, antibiotic therapy, and regular examination for treatment of the disease. However, the slaughter of infected dogs by regular examination seems to be more appropriate for the purpose of eradicating brucellosis in breeding kennels.

## REFERENCES

- Carmichael LE, Kenney RM. 1968a. Canine abortion caused by *Brucella canis*. *J Am Vet Med Assoc* 152: 605-616.

- Carmichael LE, Bruner DW. 1968b. Characteristics of a newly-recognized species of *Brucella* responsible for infectious canine abortions. *Cornell Vet* 48(4): 579-592.
- Clinical and Laboratory Standard Institute. 2007. *Performance standards for antimicrobial disk susceptibility tests: M2-A9*.
- Kim JW, Lee YJ, Tak RB. 2003a. Occurrence of canine brucellosis in large kennels and characterization of *Brucella canis* isolates by PCR-RFLP. *Korean J Vet Res* 43(1): 67-75.
- Kim JW, Lee YJ, Tak RB. 2003b. Antimicrobial susceptibility of *Brucella canis* isolated from Korea. *J Vet Clin* 20(1): 86-90.
- Kim SG, Kim YH, Park IH, Jang SJ, Jo GH, Lee YS. 2006. Two cases of antibiotic therapy in dogs infected with *Brucella canis*. *Korean J Vet Res* 29(1): 47-53.
- Kim SG, Kim YH, Hong HP, Eom HJ, Jang SJ, Jo MH, Lee YS. 2007. Biochemical characterization and PFGE pattern of *Brucella canis* isolated from kennels in Gyeongbuk province. *Korean J Vet Serv* 30(3): 363-374.
- Koh BRD, Na HM, Jang MS, Kim JY, Park SD. 2007. Investigation of canine dirofilariasis and brucellosis in free roaming dogs from public animal shelters in Gwangju area. *Korean J Vet Res* 30(1): 155-164.
- Mateu-de-Antonio EM, Martín M, Casal J. 1994. Comparison of serologic tests used in canine brucellosis diagnosis. *J Vet Diagn Invest* 6: 257-259.
- Mateu-de-Antonio EM, Martín M. 1995. *In vitro* efficacy of several antimicrobial combinations against *Brucella canis* and *Brucella melitensis* strains isolated from dogs. *Vet Microbiol* 45: 1-10.
- Moon JS, Oh GS, Park IC, Kang BK, Lee CY, Jung SC, Park YH, Shin SJ. 1999a. Therapeutic value of antibiotics in dogs infected with *Brucella canis*. *Korean J Vet Res* 39(6): 1106-1111.
- Moon JS, Oh GS, Park IC, Kang BK, Lee CY, Jung SC, Park YH, Shin SJ. 1999b. Occurrence of canine brucellosis in a large kennel in Chonnam area. *Korean J Vet Res* 39(6): 1099-1105.
- Nicoletti P. 1991. Further studies in the use of antibiotics in canine brucellosis. *The Compendium* 13: 944-947.
- Park CK, Oh JY. 2001. Bacteriological and serological investigation of *Brucella canis* infection of dogs in Taegu city, Korea. *Korean J Vet Res* 41(1): 67-71.