

Canine Brucellosis in the Jindo

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진돗개에서 발생한 부루셀라병

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요 약 : 전라남도 진도군 내에서 사육되는 진돗개에서 발생한 유산의 일부가 *Brucella canis*에 의하여 유발된 것임을 확인하였다. 이 연구에서는 먼저 1997년 7월에서 1998년 6월 사이에 발생한 진돗개의 유산을 설문조사로 확인하였던 바 발생률이 18.5%에 달했다. 이어서 설문조사에 포함된 지역에서 사육되는 진돗개를 대상으로 실시한 혈청학적 조사에서는 25%가 양성을 보였으며, 수개의 양성률은 암개의 거의 두 배에 달했다. 다음으로 균 분리와 치료시험을 실시하기 위하여 유산 병력을 가지고 있으면서 혈청검사서 양성을 나타낸 암개 다섯 마리를 현지에서 구입하였다. 그중 세 마리는 안락사 시킨 후 부검하여 자궁을 검사하고 균 분리를 시도한 바 세 마리 모두에서 *B. canis*가 분리되었다. 그러나 육안적 및 조직학적 변화는 현저하지 않았다. 나머지 두 마리는 minocycline과 streptomycin으로 치료하였는데, 치료를 끝낸 후 8주에 실시한 혈청검사서 음성을 보였다. 이 연구의 결과는 개 부루셀라병이 진돗개를 사육하는 농가에 경제적 손실을 초래하고 있음을 나타내준다. 아울러 대부분의 진돗개가 방사되고 있으며, 그렇게 사육된 개들이 전국적으로 팔려나가고 있기 때문에 진돗개가 개 부루셀라병의 전파원으로 작용할 가능성을 지니고 있음을 나타내준다. 천연기념물인 진돗개의 보호와 농가의 소득 그리고 공중위생학적인 면에서 진돗개의 부루셀라병에 관한 연구는 지속적으로 수행되어야 할 것으로 사료된다.

Key words : brucellosis, dog, the Jindo, serology, bacterial isolation, treatment.

Introduction

During the mid to late 1960s, *Brucella canis* was recognized and linked to abortions and reproductive disorders of dogs^{2,3,5}. It was found later that *B. canis* has a limited host range. Wild canids and cats are susceptible to infections, while ruminants and swine are resistant to infection⁸. Domestic dogs appear to be the definitive host of the organism.

Most dogs infected with *B. canis* show no clinical signs. After the organism penetrates a mucous membrane, it may be taken to regional lymph nodes by lymphatic drainage or it may be phagocytized by macrophages, in which it multiplies as an intracellular

parasite. By this way the infection with *B. canis* becomes systemic, but infected dogs are not usually systemically ill. Primary signs are abortion without premonitory signs during the last trimester of pregnancy, typically at 45-59 days of gestation, stillbirths, and conception failures in female dogs⁵. Male dogs may have scrotal dermatitis, testicular atrophy, epididymitis, and/or prostatitis^{2,10,20}.

The transmission of *B. canis* occurs most commonly by ingestion or inhalation of contaminated materials, although congenital and venereal infections have been verified. Infected bitches shed the organism in postgestational vaginal secretions, in milk, or in vaginal secretions during estrus. Infected males shed large numbers of organisms in the seminal fluid, rendering venereal transmissions from males to

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females possible. Infected animals commonly have prolonged bacteremia, becoming potential carriers for life. Thus, canine brucellosis caused by *B. canis* has been recognized as an important economic problem in dog breeding kennels.

The Jindo, Korean native dog, is well-known for its hunting and guarding capabilities. Unlike several other dog breeds, however, the Jindo was not developed for those purposes. The dog has been living with Korean people from the time unknown¹⁴. They have been bred in a large closed colony, known as the Jindo County for many years. More attention has been given to the dog only during the last decade¹³.

Canine brucellosis appears to be rare in Korea. In 1972, *B. suis* was isolated from an aborted fetus of a four-year old German Shepherd, and the authors postulated that the infection of the dog was caused by the contact with an infected pig²³. Recently, Moon *et al*¹⁶ found that one out of 50 Jindo and eight out of 100 mongrels were positive for *B. canis* by rapid slide agglutination test. Later, they reported the occurrence of canine brucellosis in a large kennel in Chonnam area¹⁷ and the results of antibiotics therapy in the dogs infected with *B. canis*¹⁸.

In the present study, the prevalence of abortion in the Jindo was surveyed by questionnaire. Then, the prevalence of antibodies to *B. canis* in the Jindo was ascertained by serology. Microbiological and pathological examinations were performed on three female Jindoes which experienced abortion, and two female Jindoes naturally-infected with *B. canis* were treated with antibiotics.

Materials and Methods

Field survey

A questionnaire was prepared and sent to Jindo owners in the five myons (Jindo, Gunnae, Euishin, Chodo, and Imhoe) of the Jindo County. The following information over the period of one year (from July 1997 to June 1998) was sought⁵:

- 1) The address of the owner.
- 2) The type of raising the Jindo.
- 3) If abortions occurred, the age of bitch (es) that aborted.

4) How many successive abortions were observed in individual bitches?

5) The season when the abortion occurred.

6) The size of the aborted fetuses.

7) The general condition of the bitches at the time of abortion.

Replies to this questionnaire were received from 249 Jindo owners. The bitches were one to six years old and had at least one pregnancy.

Serology

Blood samples were collected from the cephalic vein of 396 Jindoes (333 bitches and 63 dogs) in five myons, where the questionnaires were sent to the Jindo owners. The sampling was done over the period from May to October, 1998. The blood was allowed to clot at the ambient temperature. Nonhemolyzed serum was separated and frozen at -20°C until use. Serum samples were tested by Canine Brucellosis Antibody Test Kit (D-Tec CB, Synbiotics Corporation, San Diego, California), which is employing both rapid slide agglutination test (RSAT) and 2-mercaptoethanol-rapid slide agglutination test (2-ME-RSAT)⁴, as described in the instructions for the test kit. The results were recorded within 2 minutes after the card was gently rocked for 10-15 seconds and placed on a flat surface. Sera giving strong agglutination within 30 seconds were recorded as 3+, those giving agglutination within one minute as 2+, and those giving weak agglutination within 2 minutes as 1+. Only if a serum tests positive by these two test procedures should the animal be presumptively diagnosed as having *B. canis* infection.

Bacteriologic and pathologic procedures

Three female Jindoes which have histories of abortion and which were seropositive to *B. canis* were purchased from the Jindo County. The three bitches 2-3 years old were euthanized with phenobarbital sodium and necropsied. The uteri were examined grossly. For bacterial isolation, their uterine tissues were obtained. Trimmed tissues were incised several times, placed into an 11×15 cm sterile bag with an approximately equal volume of sterile saline solution, and macerated in a blender. They were inoculated into

trypticase soy broth (TSB, BBL) and cultured at 37°C for 3-7 days. Using a sterile swab, the samples were again inoculated on the blood agar plates enriched with 5% seronegative sheep blood. Identification and biotyping of suspect colonies were carried out, as described previously²¹.

For histopathological examination, tissue specimens were collected from the uteri and placed in neutral-buffered 10% formalin. The specimens were embedded in paraffin, cut into thicknesses of 5 µm, and stained with hematoxylin and eosin.

Antibiotic therapy

Two female Jindoos which have histories of abortion and which were seropositive to *B. canis* were used for antibiotic therapy. Both of them were from the Jindo County, and treated with minocycline 12.5 mg/kg PO BID for 4 weeks and streptomycin 5 mg/kg IM BID for one week¹⁰. Eight weeks after the termination of the therapy, serum samples of the two bitches were tested for *B. canis* antibodies.

Results

Field data

It was found by questionnaire that 46 female Jindoos (18.5%) out of 249 have had abortions. Most (36 bitches) of the Jindoos which had abortions were raised unleashed in the farmhouse as part of a family; seven bitches were from the farmhouses which have both breeding kennels and dogs unleashed, while the rest three bitches were from breeding kennels.

No trends were shown in the abortion rates with age, although the two-year-old group had the highest (22.7%) abortion rate. The abortion rate was highest (45.7%) in summer, and of the fetuses aborted, about 95% were longer than 6 cm in the size of crown-rump length.

Serology

Of the 396 Jindoos tested serologically, 99 (25%) showed positive reactions to *B. canis* by both RSAT and 2-ME-RSAT (Table 1). Of those 99 Jindoos, 15 showed 1+, 40 2+, and the rest 44 3+. The seropositive rate was highest (52.5%) in two-year-old age group and decreased with age. The seropositive rate

Table 1. Serosurvey of 396 Jindoos for *Brucella canis* by the rapid slide agglutination test and 2-mercaptoethanol rapid slide agglutination test

	No. of Jindoos examined	Rate (%)
Negative	297	75.0
1+	15	3.8
2+	40	10.1
3+	44	11.1

Canine Brucellosis Antibody Test Kit (D-Tec CB, Synbiotics Corporation, San Diego, California) was used for the tests. Sera giving strong agglutination within 30 seconds were recorded as 3+, those giving agglutination within one minute as 2+, and those giving weak agglutination within 2 minutes as 1+.



Fig 1. Uteri of a Jindo infected with *Brucella canis*, showing dark-brown spots (arrows) on the mucosa.

of dogs (26/63 dogs) was almost twice that of bitches (73/333 bitches).

Pathological findings

On gross examination, lesions were found in the two female Jindoos, in which dark-brown spots were noted on the mucosal surface of the uteri (Fig 1). No lesions were found in one bitch. Histopathological examinations of the uteri of the two bitches revealed mild lymphocytic infiltrates in the submucosa.

Bacterial isolation

Bacteria were isolated from the two bitches which had the uterine lesions. The bacterial colonies were whitish-grey and transparent, and 0.3-2 mm in diameter. They were identified as *B. canis* by their biochemical characteristics.

Antibiotic therapy

The two female Jindoos treated with minocycline and streptomycin showed complete remission. No antibodies were detected in their sera after the treatment, and they were pregnant after one year and delivered pups naturally.

Discussion

In the present study, the presence of canine brucellosis was verified in Jindoos reared in the Jindo County. Canine brucellosis should be suspected whenever apparently healthy bitches abort approximately 2 weeks before parturition⁵. It was found by questionnaire that about 20% of female Jindoos, which were healthy otherwise, experienced abortions. In the subsequent serological work performed in Jindoos of the same area, 25% of Jindoos both sexes showed positive reactions to *B. canis*. *B. canis* was isolated from three female Jindoos, which have histories of abortion and which were seropositive to *B. canis*. These results suggest that canine brucellosis caused by *B. canis* has been a major source of economic loss in the Jindo.

The Jindo is one of the sources of household income for the farmers in the Jindo County. The dog has been maintaining its original qualities because of the geographical location of the Jindo County and of the law enforced by the local government since early 1960s¹³. The dog has been bred in a large closed colony, known as the Jindo County for many years. The County consists of a total of 228 islands, with its main island of about 427 square kilometers. The population of the Jindo in the entire County reaches 12,000 each year, and about 3,000 Jindoos are taken out of the County every year¹². It was estimated that the number of Jindoos which are being kept outside the County could be in the neighborhood of 160,000²⁴. These indicate the importance of canine brucellosis caused by *B. canis* in the Jindo.

In the old days, almost all of Jindoos were reared unleashed in the farmhouse as part of a family. In a survey conducted in 1986¹¹, more than half of Jindoos were being reared in such a fashion, free roaming; male Jindoos were usually not leashed. It was found

in this study that 36 out of 46 Jindoos which have had abortions were free roaming and that the seropositive rate of dogs was almost twice that of bitches. These results support the previous findings^{1,6,7,9,15}, in which the prevalence of positive serologic results in free roaming (or stray) dogs was considerably higher than in pet (or confined) dogs.

In the present study, 25% of Jindoos showed seropositive reactions to *B. canis*, which is much higher than the rates reported previously^{1,6,7,15}. In adult dogs, *B. canis* infection is characterized by few clinical signs. Transient lymphadenopathy occurs early in the course of infection, but it is usually undetected by the owner. Fever is rare. The most noticeable clinical signs of canine brucellosis are abortion during the third trimester in bitches and physical changes within the scrotum in dogs. These signs occur in an otherwise healthy dog, and are usually not detected by the owner, especially in the free roaming dogs^{10,20}. However, bacteremia is usually detectable two to four weeks after the dog is infected and can persist for 6 to 64 months, thus becoming potential carrier for life¹⁰. These explain the reason why the Jindo showed exceptionally high seropositive reactions to *B. canis* and indicate the necessity of further study on the canine brucellosis in the Jindo.

Antimicrobial chemotherapy for *B. canis* infections is frequently unsuccessful, as is typical with infections caused by intracellular organisms. It was reported that a combination of minocycline and streptomycin is most successful treatment for canine brucellosis^{10,18}. The treatment regimen was applied to two female Jindoos infected with *B. canis* in the present study, and the treatment was found to be successful both serologically and clinically.

There is no immunizing agent that protects dogs against systemic infection with *B. canis* at the present time. The large closed colony of the Jindo in the Jindo County can be regarded as a kennel, and there are a few reports on the eradication or control of *B. canis* in a kennel^{10,19,22}. From the results in the present study, it is indicated that canine brucellosis in the Jindo should be controlled by the following procedures: confirmation of the diagnosis, quarantine of the County, determination of the source of infection,

elimination of the mode of transmission in the County, identification and elimination of infected animals, and initiation of practices to prevent future outbreaks. The disease can be controlled and eradicated. However, these procedures are quite costly in both time and materials and should be implemented and vigorously continued until the infection is eradicated.

Conclusions

The presence of canine brucellosis caused by *B. canis* was verified in Jindo dogs reared in the Jindo County. It was found by questionnaire that about 20% of female Jindo dogs experienced abortions. By the serological work performed in Jindo dogs of the same area, 25% of Jindo dogs both sexes were shown to be positive to *B. canis*. *B. canis* was isolated from female Jindo dogs, which have histories of abortions, and female Jindo dogs infected with *B. canis* were treated with minocycline and streptomycin. Control of the infection was discussed with regard to the previous experiences elsewhere.

References

1. Brown J, Blue JL, Wooley RE, Dreesen DW. *Brucella canis* infectivity rates in stray and pet dog populations. *Am J Publ Hlth* 1976; 66: 889-891.
2. Carmichael LE. Abortion in 200 beagles. *J Am Vet Med Assoc* 1966; 149: 1126.
3. Carmichael LE, Bruner DW. Characteristics of a newly-recognized species of *Brucella* responsible for infectious canine abortions. *Cornell Vet* 1968; 58: 579-592.
4. Carmichael LE, Joubert JC. A rapid slide agglutination test for the serodiagnosis of *Brucella canis* infection that employs a variant (M-) organism as antigen. *Cornell Vet* 1987; 77: 3-12.
5. Carmichael LE, Kenney RM. Canine abortion caused by *Brucella canis*. *J Am Vet Med Assoc* 1968; 152: 605-616.
6. Fredrickson LE, Barton CE. A serologic survey for canine brucellosis in a metropolitan area. *J Am Vet Med Assoc* 1974; 165: 987-989.
7. Galphin SP. A serologic survey for *Brucella canis* in dogs on a military base. *J Am Vet Med Assoc* 1977; 171: 728-729.
8. Hoff GL, Bigler WJ, Trainer DO, Debbie JG, Brown GM, Winkler WG, Richards SH, Reardon M. Survey of selected carnivore and opossum scrums for agglutinins to *Brucella canis*. *J Am Vet Med Assoc* 1974; 165: 830-831.
9. Hubbert NL, Bech-Nielsen S, Barta O. Canine brucellosis: comparison of clinical manifestations with serologic test results. *J Am Vet Med Assoc* 1980; 177: 168-171.
10. Johnson CA, Walker RD. Clinical signs and diagnosis of *Brucella canis* infection. *Compend Contin Educ Prac Vet* 1992; 14: 763-773.
11. Kim YK, Kim JH, Lee CG, Lee CY, Park GS. Studies on the preservation of the Jindo dogs - a baseline examination. The Jindo County Research Report. 1986.
12. Lee CG. Preservation and rearing of Korean Jindo dogs. *Proc 4th Ann Meeting, Korean Fed Soc Anim Sci* 1994; 82-89.
13. Lee CG, Lee JI, Lee CY, Sun SS. A review of the Jindo, Korean native dog. *Asian-Aust J Anim Sci* 2000; 13: 381-389.
14. Lee CG, Yoo GH. Jindo dogs-Korean native dogs: A historical review. *Korean J Vet Res* 1988; 28: 405-408.
15. Lovejoy GS, Carver HD, Moseley IK, Hicks M. Serosurvey of dogs for *Brucella canis* infection in Memphis, Tennessee. *Am J Publ Hlth* 1976; 66: 175-176.
16. Moon JS, Park YH, Jung SC, Ku BG, Jang GC, Shin S, Lee SI, Lee JM, Shin SJ. Studies on serological tests in canine brucellosis. *RDA J Agri Sci* 1994; 36: 614-621.
17. Moon JS, Oh GS, Park IC, Kang BK, Lee CY, Jung SC, Park YH, Shin SJ. Occurrence of canine brucellosis in a large kennel in Chonnam area. *Korean J Vet Res* 1999; 39: 1099-1105.
18. Moon JS, Oh GS, Park IC, Kang BK, Lee CY, Jung SC, Park YH, Shin SJ. Therapeutic value of antibiotics in dogs infected with *Brucella canis*. *Korean J Vet Res* 1999; 39: 1106-1111.
19. Moore JA, Gupta BN, Conner GH. Eradication of *Brucella canis* infection from a dog colony. *J Am Vet Med Assoc* 1968; 153: 523-527.
20. Moore JA, Kakuk TJ. Male dogs naturally infected with *Brucella canis*. *J Am Vet Med Assoc* 1969; 155: 1352-1358.
21. Nicoletti P. *Brucella*. In: Carter GR, Cole JR, editors. *Diagnostic procedures in veterinary bacteriology and mycology*, 5th ed. San Diego: Academic Press. 1990: 95-105.
22. Pickerill PA, Carmichael LE. Canine brucellosis: control programs in commercial kennels and effect

- on reproduction. J Am Vet Med Assoc 1972; 160: 1607-1615.
23. Tak RB, Chun DK. Isolation of *Brucella suis* from aborted fetus of a dog. J Kor Soc Microbiol 1972; 7: 17-20.
24. Yoon SK. Jindo dogs. Seoul: Jisik Seokwan, 1997 81.