東京地区のイヌにおけるBrucalla canisの汚染調査

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A Survey of *Brucella canis* Infection in Dogs from Tokyo Area

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Abstract. A total of 945 adult dogs, 572 stray and 373 nonstray ones, collected from Tokyo area, were examined for *Brucella canis* infection and 27 (2.9%) cases were found to have high titered agglutinin and/or the organism. With the exception of a colony of nonstray dogs showing a positive rate of 12.7%, no significant difference in positivity rates was seen between stray and nonstray dogs. Bacteremia was detected in 12 cases out of 459 cases examined, and 9 positive cases had high titered antibodies. In 9 out of 11 autopsied cases having high levels of agglutinin or positive blood culture, the organism was isolated from the spleen, lymph nodes, liver and male genital organs with high frequency. Among these cases carrying the organism, high titered specific agglutinins were usually resistant to 2-mercaptoethanol treatment, while low titered agglutinins, probably nonspecific, were sensitive.

In Japan, canine brucellosis due to *Brucella canis* (*B. canis*) was first recognized in 1972 in a beagle breeding colony established originally by dogs introduced from the United States [24]. In 1973, however, another epidemic occurred in a dog colony in Tokyo involving not only imported beagles but also stray dogs collected from Tokyo and Saitama Prefectures [10]. The presence of *B. canis* infection was further detected in both stray and pet dogs in Tokyo area [19]. This report deals with the results of a larger scale of survey for the prevalence of *B. canis* infection among native dogs made during a period from April 1974 to July 1977.

Materials and Methods

Dogs: A total of 945 dogs, 572 stray and 373 nonstray, from 6 sources as presented in Table 1 were examined. Of the 572 stray dogs, 429 were collected in Tokyo Prefecture and introduced to this institute for experiments, and 143 were collected in Tokyo and Chiba Prefectures and introduced to Tokyo University Veterinary Hospital for experimental use. Of the 373 nonstray dogs, 175 were pet dogs introduced to an animal shelter in Kanagawa Prefecture for euthanasia, 87 were from 2 training schools, A and B located in Tokyo, and 111 were patient dogs at Tokyo University Veterinary Hospital and practicing veterinarians in Tokyo.

Most dogs examined were mongrel except for some Shepherd, Doberman-pinscher, Duckshund and Akita breeds. The age and sex were not exactly known in many cases, but all of them were adult males and females, nearly equal in number.

Serology: Blood samples were obtained from the cephalic vein and sera were stored at −20°C before testing. From April 1974 to April 1975, a tube agglutination test was made according to a modification of Carmichael's one using an antigen prepared from strain RM-666 [18]. In this private method, to 0.5 m*l* of serially diluted sera was added 1 m*l* of

東京地区のイヌにおける *Brucella canis* の汚染調査：三枝順三・上田雄幹・後藤義孝・藤原公策（東京大学医科学研究所獣医学研究部）

antigen suspension, and agglutinin titer was expressed by the highest serum dilution before adding the antigen, which gave a complete agglutination. Sera showing complete agglutination at 1:200 or higher were considered positive.

Since May 1975, the private method has been replaced by a standard method using an antigen prepared from strain QE-13, the first isolate in Japan [1], according to a proposal of Japan Brucellosis Center. For this standard method, a concentrated antigen was provided by the courtesy of Dr. Ghoda at Kitasato Institute, Tokyo. The method of preparation of this type of antigen was the same as described for that of RM-666 [18]. Before use, the concentrated antigen was diluted with phosphate-buffered saline (pH 7.2) to give a transparency of 0.4 at 420 nm determined by a spectrophotometer. To 0.5 m*l* of serial twofold dilutions of test sera starting at 1:10 was added an equal volume of the diluted antigen suspension and the mixtures were incubated at 50 to 52°C for 48 hr. In this case, agglutinin titer was expressed by the final serum dilution after adding the antigen. Comparative testing between the private method and the standard one was made repeatedly, revealing that a titer of 1:640 in the standard method was almost equivalent to that of 1:200 in the private one. Then, in this paper, the titers according to the private method were converted to equivalent titers of the standard method.

To see sensitivity of agglutinin to 2-mercaptoethanol (2 ME) [16], each serum sample was incubated with an equal volume of 0.2 M 2 ME at 37°C for 1 hr and then tested by the standard tube agglutination method without elimination of 2 ME.

Bacteriology: A 0.1 m*l* amount of blood taken aseptically from the cephalic vein was inoculated on Tryptic soy agar (TSA) (Difco) plates usually in duplicate. In case of autopsied dogs, 1 m*l* blood was also inoculated into 100 m*l* of Trypticate soy broth (BBL) in a bottle without adding anticoagulant. These inoculated plates and bottles were incubated aerobically at 37°C for 7 days. From the broth cultures, 0.1 m*l* of each sample was taken at 3, 5 and 7 days postinoculation and inoculated on a TSA plate, which was incubated at the same condition for 3 days.

For the detection of the organism from internal organs, dogs were anesthetized by intramuscular inoculation with ketamine hydrochloride (Park, Davis and Sankyo) and bled from the carotid artery. Then the isolation of *B. canis* was made from the liver, spleen, kidney, lung, retropharyngeal, iliac and popliteal lymph nodes, urinary bladder, bladder urine, bone marrow, testicle, epididymis, prostate, uterus and vagina. The cut surfaces of these tissue specimens were stamped on TSA plates and 0.1 m*l* of urine was inoculated onto the same medium. The plates were incubated aerobically at 37°C for 7 days.

Colonies suspected of *B. canis* which had been developed on TSA were examined for Gram stain and slide agglutination with anti-*B. canis* rabbit serum [18]. The isolates suspected of *B. canis* were sent to Dr. Isayama, Japan Brucellosis Center, and all of them were identified biologically as well as serologically as *B. canis*.

Results

Prevalence of infection: Twenty-seven of 945 cases (2.9%) were considered to be infected from the detection of either serum agglutinin or *B. canis* organism from blood and/or organs (Tables 1 and 2). Among these positive 27 cases, 11 and 16 were strays and nonstrays, respectively (Table 3). The latter contained 8 out of 63 (12.7%)

Table 1. Detection of *B. canis* infection

Group	Positive*/Tested (%)
Inst. Med. Sci.	6 / 429 (1.4)
Tokyo Univ. Vet. Hosp.	6 / 203 (3.0)
Kanagawa	6 / 175 (3.4)
Training School A	8 / 63 (12.7)
Training School B	0 / 24 (0)
Others	1 / 51 (2.0)
Total	27 / 945 (2.9)

Remarks.
*: Confirmed by detection of positive agglutinin titer or *B. canis* from blood or organs.

Table 2. Relationship between serology and bacteriology

Serum agglutinin	Detection of *B. canis*	No. of case
+*	+**	13
+	—	5
+	n.t.	7
—	+	2
Total		27

Remarks.
*: (+)Titer higher than 1 : 640; (—) Titer lower than 1 : 320.
**: From blood or organs.
n.t.: Not tested.

Table 3. Positive rate of stray and nonstray dogs

Case	Positive*/Tested (%)
Stray	11 / 572 (1.9)
Nonstray	16 / 373 (4.3)
Training School A	8 / 63 (12.7)
Others excluding Training School A	8 / 310 (2.9)

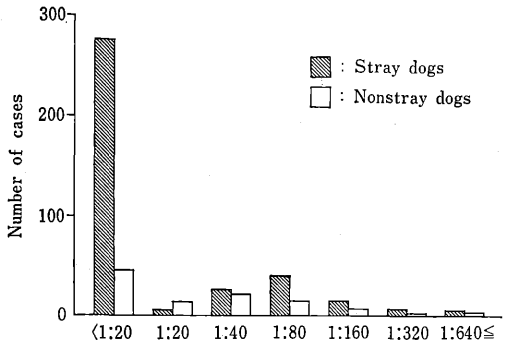
Remarks.
*: See the foot note of Table 1.

positive cases of Training School A, and the swelling of the testicle or epididymis was recognized in some male dogs. This and another School B were subjected to this survey since the infection at these training schools was suspected from a positive case at Tokyo University Veterinary Hospital. At Training School B, however, neither positive agglutinin nor *B. canis* was detected, although some dogs had been introduced from School A.

Mercaptoethanol sensitivity of agglutinin: The distribution of agglutinin titers among 490 dogs tested by the standard method is presented in Fig. 1, revealing a small peak from 1:40 to 1:160. Then, sera from cases showing low titered agglutinin and those from 11 autopsied cases were examined for 2ME sensitivity of agglutinin. The results of all the autopsied cases and some typical ones with low titered agglutinin were presented in Fig. 2. In most cases showing titers of 1:320 or lower, which were categorized as negative in this study, the agglutinins were found to be predominantly 2ME sensitive as in Cases 15 to 18, while a few cases such as Cases 6 and 14 had 2ME resistant ones. On the contrary, in dogs carrying the organism in blood or organs, agglutinins were predominantly 2ME resistant, though the low titered agglutinin of a bacteremic Case 7 was predominantly 2ME sensitive. In Cases 10 and 11 having an agglutinin at titer of 1:1280 but carrying no organism in blood and organs, antibodies were shown to be fairly 2ME sensitive. Case 13, from which bacterial culture failed to be made, was found to have 2ME sensitive agglutinin at a titer of 1:640.

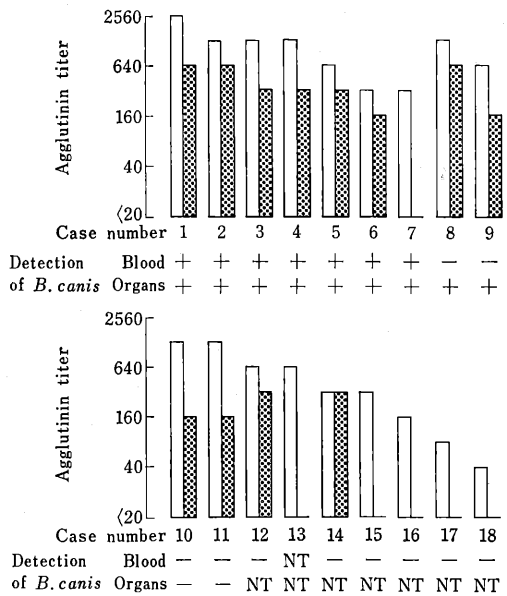
Isolation of *B. canis* from blood and organs: Blood cultures were conducted on 459 of 945 cases examined serologically. Bacteremia was detected in most cases hav-

Fig. 1. Incidence of agglutinin titer to *B. canis*



Remarks.
490 cases tested with the standard method.

Fig. 2. Mercaptoethanol sensitivity of seroagglutinin



Remarks.
□ : Total titer.
▨ : 2ME resistant titer.
NT : Not tested.

Table 4. Detection of *B. canis* from organs

Breed	Sex	Agg. titer at autopsy	Organs																	
			Blood	Liver	Kidney	Lung	Spleen	Retroph. node	Iliac node	Popli. node	Bone Marrow	Urin. Bladder	Blad. Urine	Prostate	R. Epididym.	L. Epididym.	R. Testicle	L. Testicle	R. Uterus	L. Uterus	Vagina
Mongrel	Male	1 : 1280	—	—	—	—	—	—	—	—	—	—	—	++	—	—	—	—	•	•	•
Shepherd	Male	1 : 1280	+	++	—	—	+++	+++	+++	+++	+	—	—	++	+	++	—	—	•	•	•
Mongrel	Male	1 : 1280	++	++	—	+	++	++	+	—	++	—	—	—	—	—	—	—	•	•	•
Shepherd	Female	1 : 320	+	+	+	—	++	++	++	+	—	—	++	•	•	•	•	•	—	—	+
Mixed-Akita	Male	1 : 1280	—	+	—	—	++	++	++	++	—	C	—	+	—	—	—	—	•	•	•
Mongrel	Male	1 : 640	+	++	—	—	+++	+++	+++	+++	++	+++	+++	++	++	C	++	+++	•	•	•
Mongrel	Male	1 : 1280	+	+	—	—	+++	++	++	++	—	—	+	++	++	++	+	—	•	•	•
Mongrel	Male	1 : 1280	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	•	•	•
Mongrel	Male	1 : 2560	+°	++	C	—	++	+++	++	+++	++	C	C	+++	—	—	—	+	•	•	•
Mongrel	Female	1 : 1280	—	—	—	—	—	—	—	—	—	—	—	•	•	•	•	•	—	—	—
Mongrel	Female	1 : 320	+	++	+	+	+++	+++	+++	+++	+	—	+	•	•	•	•	•	—	—	—

Remarks.

— : Not detected.
+ : 1–9 colonies per plate.
++ : 10–99 colonies per plate.
+++ : 100–999 colonies per plate.
+++ : More than 1000 colonies per plate.
C : Contaminated.
+° : Detected by broth culture.

ing serum agglutinin titers of 1:1280 or over. However, two cases having an antibody titer of 1:1280 were nonbacteremic while 2 seronegative cases, of which titer was 1:320, were bacteremic.

Eleven of 27 cases having either seropositivity or bacteremia were autopsied. No organism was detected from 2 seropositive but nonbacteremic cases, while the organism was isolated from the remaining 9 cases (Table 4). The prevalence in detection of the organism was seen from the spleen, retropharyngeal, iliac and popliteal lymph nodes, liver, prostate and blood. The organism was also isolated from the vagina of one of 3 females examined.

Of 2 cases which were euthanatized in a CO_2 gas chamber after sampling blood for serological examination at an animal shelter in Kanagawa Prefecture, only the testicle, epididymis and mesenteric lymph nodes having been stored at −20°C were subjected to the isolation of *B. canis* after seropositivity was determined. The results were positive in the mesenteric lymph nodes in one of the 2 cases.

Discussion

Canine brucellosis has been recognized mostly in beagle colonies in the United States [3, 8, 13], West Germany [21, 23] and Japan [24]. However, serosurveys in some countries [2, 4–6, 9, 11,12] revealed that the infection is prevalent also in native dogs, and *B. canis* was isolated from pets [15, 17] or native stray dogs [5].

The presence of *B. canis* infection among stray and nonstray dogs in Tokyo area [19] was further confirmed by the present study. The positivity rate of 2.9% is in agreement with the results of surveys made in other parts of Japan, Sapporo [7] and Kyoto (Dr. T. Serikawa, personal communication), but seems to be lower than those reported in

other countries [2, 4–6, 9, 11, 12].

An extremely high positivity rate at a dog training school was of interest, suggesting an important role of such facilities for transmission of the disease among nonstray dogs. Excluding this case, no significant difference was seen between the positivity rates of stray and nonstray dogs in the present study, while positivity rate in stray dogs has been reported to be higher than that of nonstray ones in the United States [2, 6, 11].

Positive culture from internal organs was frequently observed in dogs having high titered antibodies, indicating that agglutinin titers higher than 1:640 in the standard method may reflect persistent infection of *B. canis*. Bacteremia was usually detected in dogs having agglutinin titers higher than 1:1280 in the standard method, thus the co-existence of bacteremia and high agglutinin titer was evidenced as reported by others [3, 14, 20]. However, 2 cases having an agglutinin of 1:1280 showed no bacteremia and other 2 seronegative cases showed bacteremia as reported by von Kruedenner [22]. Then, the definitive diagnosis for persistent infection should be made by isolation of the organism from blood or organs.

A small peak in the titer distribution was recognized at a range of 1:40 to 1:160 by the standard method. The same tendency was said to be noticed in a survey in Kyoto (Dr. T. Serikawa, personal communication). Such low titered agglutinin, probably nonspecific, was shown to be predominantly 2ME sensitive as shown in Fig. 2, while high titered agglutinin detected in *B. canis* carrying cases was mostly 2ME resistant. In this regard, the application of 2ME treatment to reduction of nonspecific activity might be reasonable as proposed by Biological Reagents Section, Veterinary Services Diagnostic Laboratory, the United States Department of Agriculture, Ames, Iowa [9]. In such treatment, however, some cases at the early phase of the infection may be missed. In the 2 exceptional cases mentioned already, the agglutinins were shown to be 2ME sensitive and these were considered to be convalescent cases as described by van Hoosier [20].

B. canis was isolated from 9 of 11 dogs autopsied, and relatively high positivity were observed in the lymphoreticuler organs, blood and male genital organs. This is in agreement with the results of our previous report [18] as well as those reported by Carmichael [3], Moore [14] and van Hoosier [20].

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