An Epidemiological Survey of *Brucella canis* Infection of Dogs in the Towada Area of Aomori Prefecture

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Brucella canis infection was first recognized in the United States by Carmichael in 1966 [3]. In Japan, canine brucellosis due to *B. canis* was first recognized in a beagle breeding colony in 1972. Since 1973, many investigators have studied canine brucellosis epidemiologically in Japan; in dogs of the Tokyo metropolitan area [15, 20], Hokkaido [8], Gifu and Shiga prefectures [17], Kyushu area [22], Tohoku area [16], and Ibaraki prefecture [23]. However, no epidemiological survey of this disease in dogs has been conducted anywhere in Japan since 1983. We noticed epididymitis in Tosa-breed in the Towada area, Aomori prefecture in October 1989. The possibility for the outbreak of canine brucellosis existed; therefore, an epidemiological survey was made over the period from October 1989 to August 1990 to determine the prevalence of agglutinins to *B. canis* in dogs of the Towada area.

We investigated 259 dogs consisting of 142 Tosa-breed in the Towada area and 117 captured dogs in health centers in Towada, Misawa and Shichinohe. Blood samples were obtained from a vein of the foreleg of the Tosa-breed and directly from the heart of captured dogs after euthanasia by strychinine or succinylcholine. All blood samples were treated with heparin solution and the one part was reserved into a culture bottle with Tryptose broth (Difco) and the plasma was separated from heparinized blood for agglutination test. Agglutination tests were conducted with an antigen prepared from strain QE-13, the first isolate in Japan [1, 2, 12], according to a proposal of the Japan Brucellosis Center. For the standard method, a concentrated antigen was provided by Kitasato Institute Hospital, Tokyo. The concentrated antigen (QE-13 antigen) was diluted with phosphate-buffered saline (pH 7.2) to give an optical density (OD) of 0.54 at 620 nm. The equal volume of the diluted antigen was added to 0.5 m*l* quantities of serial twofold dilutions of test sera starting at 1:10 and the mixtures were incubated at 50°C for 24 hr. The agglutinin titer in the test tube method was expressed as the final serum dilution givin 50% agglutination and sera with a titer of 1:160 or more were considered positive. We compared the modified microtiter method repeatedly based on those Damp *et al.* [6] and Serikawa *et al.* [18] with the standard method, using anti-*B. canis* QE-13 rabbit serum (positive antiserum for reference: agglutinin titer 1:320) and the QE-13 antigen. The optimum conditions for the microtiter method which gives the same titers as the standard method were determined as follows: The volumes of antigen and antiserum were 50 μ*l*/well each. The optimum concentration of the QE-13 antigen was obtained at OD 0.84 at 620 nm. None of the serum or plasma samples were inactivated. The agglutination test was performed at 50°C for 24 hr. Strychinine and succinylcholine which were used

Table 1. Agglutinin titers[a)] against *Brucella canis* and isolation[b)] of the organism

Serum (Plasma) dilution	Agglutinin titer: Tosa-breed (%)		Dogs from various area: Towada (%)		Misawa (%)		Shichinohe (%)		Total (%)		Isolation: Number of dogs with *B. canis* (%)	
<1:20	109	(76.8)	48	(85.7)	30	(96.8)	24	(80.0)	211	(81.5)	ND[c)]	
1:20	19	(13.4)	2	(3.6)	0	(0.0)	3	(10.0)	24	(9.3)	0/24	(0.0)
1:40	10	(7.0)	4	(7.1)	0	(0.0)	0	(0.0)	14	(5.4)	0/14	(0.0)
1:80	3	(2.1)	1	(1.8)	0	(0.0)	1	(3.3)	5	(1.9)	0/ 5	(0.0)
1:160	1	(0.7)	0	(0.0)	1	(3.2)	2	(6.7)	4	(1.5)	0/ 4	(0.0)
1:320	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0/ 0	(0.0)
1:640	0	(0.0)	1	(1.8)	0	(0.0)	0	(0.0)	1	(0.4)	1/ 1	(100.0)
Positive (%)	1	(0.7)	1	(1.8)	1	(3.2)	2	(6.7)	5	(1.9)	1/ 5	(20.0)
Total	142		56		31		30		259		1/48	(2.1)

a) Microtiter method using strain QE-13 antigen.
b) Blood culture on Tryptose Agar.
c) Not done.

to kill the captured dogs did not interfere with the agglutination. The agglutinin titer in the microtiter method was expressed as the final dilution giving complete agglutination. In this paper, All agglutinin titers were obtained from the microtiter method.

The agglutinin titers of the Tosa-breed and the captured dogs in the three health centers of the Towada area are summarized in Table 1. Five of the 259 dogs (1.9%) were positive for agglutinins (1:160 in 4 cases and 1:160 in 1 case). Of the 142 Tosa-breed, one was positive (0.7%). Of the 117 dogs in the three health centers, 4 dogs were positive (3.4%). Three of these 4 had agglutinin titers of 1:160 and one had 1:640. A blood culture was made on 48 dogs (agglutinin titer 1:20 or more) of the 259 dogs by aerobic culture on Tryptose agar (Difco) at 37°C for 48 hr. *B. canis* was isolated from one captured dog which showed an agglutinin titer of 1:640. Biological characteristics of the isolated organism were compared with those of the reference strain QE-13. The results are shown in Table 2. The isolated organism, designated as strain MK-4, showed the same characteristics as the reference strain. In addition, cross absorption tests were performed between the two antibodies and the two antigens; the anti-QE-13 rabbit serum (reference positive antiserum, agglutinin titer 1:320) and the plasma of the dog from which strain MK-4 was isolated (agglutinin titer 1:640) were used as antibodies and strains QE-13 and MK-4 as antigens. These cross agglutination tests showed complete cross absorption (Table 3).

Table 2. Biological characteristics of isolated organism (Strain MK-4)

Morphological and biochemical test	Result
Gram stain	−
Growth under anaerobic condition	−
Growth on MacConkey's agar	−
Catalase production	+
Oxidase production	+
Motility[a]	−
Indole production[a]	−
H_2S production[a]	−
Citrate utilization[b]	−
Voges-Proskauer test[b]	−
Gelatin hydrolysis[b]	−
Acid production[b]	
Glucose	−
Mannitol	−
Inositol	−
Sorbitol	−
Rhamnose	−
Sucrose	−
Melibiose	−
Amygdalin	−
Arabinose	−
Urease production[b]	+
Nitrate reduction[b]	+
Agglutination in acriflavin solution	+

a) SIM medium.
b) API-20E system.

Ueda *et al.* [21], in 1974, reported a positive rate of 3.6% (4/112) in dogs caputured in an urban area of Tokyo. Serikawa *et al.* [17] made a survey from April 1976 to March 1977 in Gifu and Shiga prefectures and reported that the positive rates were 2.5% (21/847) in Gifu and 3.5% (12/339) in Shiga prefectures. Hayashi and Isayama [8] surveyed dogs in Hokkaido from April 1974 to March 1975, and reported a positive rate of about 3% (16/540). Saegusa *et al.* [15] found a positive rate of 2.9% (27/945) in the Tokyo area from April 1974 to April 1975. In Kyushu, Wada *et al.* [22] reported a total positive rate of 1.6% (27/1739) from March 1977 to October 1978, and the rates by prefecture were 3.5% (15/431) in Fukuoka, 1.7% (1/60) in Saga, 0.8% (1/130) in Nagasaki, 1.4% (2/138) in Kumamoto, 2.3% (2/88) in Miyazaki, 0% (0/170) in Kagoshima and 1.2% (2/162) in Okinawa. In the Tohoku area, Sakuma *et al.* [16] made a survey in 1977 and reported a positive rate of 7.6% (24/315). The rates in the six prefectures in Tohoku area were 8.5% (5/59) in Aomori, 7.1% (4/56) in Iwate, 9.4% (8/85) in Miyagi, 8.9% (5/56) in Fukushima, 2.6% (1/38) in Akita and 4.8% (1/21) in Yamagata. Moreover, Shirasaka *et al.* [23] made a survey from 1982 to 1983 in Ibaraki prefecture, and reported a positive rate of 6.1% (62/1010).

The results of the survey showed an average positive rate (a titer of 1:160 or more) of 1.9% (5/259). Only one dog showed an agglutinin titer of 1:640 and *B. canis* was isolated from his blood.

In this paper, we pointed out that the canine brucellosis was not free at Towada area in Aomori prefecture and need further survey of the disease in Japan.

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Table 3. Cross-agglutinin absorption test

Antibody	Antigen for absorption	Agglutinin titer		
		Before absorption	After absorption with	
			QE-13	MK-4 (the isolate)
Anti-QE-13 rabbit serum	QE-13	1:320	<1:20	<1:20
	MK-4	1:320	<1:20	<1:20
Plasma from the infected dog	QE-13	1:640	<1:20	<1:20
	MK-4	1:640	<1:20	<1:20

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