Seroprevalence of *Bartonella henselae* and *Toxoplasma gondii* Infections among Pet Cats in Kanagawa and Saitama Prefectures

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ABSTRACT. Seroprevalence of *Bartonella henselae* and *Toxoplasma gondii* was investigated among 471 pet cats obtained from seven private animal hospitals in Kanagawa and Saitama Prefectures during the period from May 1994 to June 1995. Furthermore, 67 randomly selected from the 471 serum samples were examined for the feline immunodeficiency virus (FIV) antibody and feline leukemia virus (FeLV) antigen. The antibody to *B. henselae* was examined by an indirect immunofluorecent antibody test. *T. gondii*, FIV and FeLV infections in cats were detected with respective commercial kits. Of the cat serum samples tested, 43 (9.1%) were found to be seropositive for *B. henselae* and 41 (8.7%) for *T. gondii*. The *B. henselae*-positive rate (12.9%) of male cats was significantly higher than that (5.2%) of female cats. On the other hand, *T. gondii*-positive rate was 9.1% in male and 8.7% in female cats and there was no significant difference in the positivity between sexes. The positive rate in each hospital varied from 0 to 19.5% for *B. henselae* and 4.9 to 18.8% for *T. gondii.* The ages of *B. henselae*- and *T. gondii*-positive cats were distributed from <1-year-old to 14-year-old and the seropositivity increased with age of cats. Of the 67 cat serum samples, 16 and 6 cases were positive for FIV and FeLV, respectively. There was no relationship between these viral and *B. henselae* infections in cats. — KEY WORDS: *Bartonella henselae*, cat scratch disease, FeLV, FIV, *Toxoplasma gondii*

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Cat scratch disease (CSD) in humans is a benign disease characterized mainly by pyrexia, persistent regional lymphadenopathy, and skin lesions such as papule, small vesicle, and ulcer on the site of cat scratch or bite [2, 22]. The causative agent of CSD, however, remained unknown for a long time after the disease was reported by Debré *et al.* in 1950 [8]. Recent investigations suggested that a fastidious bacterium *Bartonella henselae* is a causative agent for bacillary angiomatosis (BA) and bacillary peliosis (BP) in HIV-positive patients [18, 21, 29]. Furthermore, serological investigations strongly suggested that *B. henselae* is a causative agent of CSD [25, 31] and the organism was isolated from typical CSD patients in immunocompetent status [10].

More than seven million cats are being kept as pets in this country, and suspected human cases of CSD are reported every year [20, 26, 30]. Though cats are strongly considered to be the reservoirs of the disease, only a few reports are available on epidemiology of *B. henselae* infection in domestic cats in this country [23, 27, 28]. Therefore, prevalence of the infection among domestic cats in Japan remains till obscure.

On the other hand, *Toxoplasma gondii* is also another important zoonotic agent in cats and humans [11]. Although many epidemiological surveys for *T. gondii* in Japanese cats have been carried out in the past few decades [12, 14, 16, 17, 24], recent infectious status of the disease in pet cats has not been reported.

The purpose of this study was to determine the serological prevalence of *B. henselae* and *T. gondii* infections among pet cats in Kanagawa and Saitama Prefectures as a basic study of the epidemiology of CSD and toxoplasmosis in Japan. Furthermore, relationship among *B. henselae, T. gondii,* feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections in pet cats was also examined.

MATERIALS AND METHODS

Cat samples: During the investigation period from May 1994 to June 1995, a total of 471 serum samples were collected from four private veterinary hospitals in Kanagawa Prefecture and three hospitals in Saitama Prefecture in the Kanto District in Japan (see Table 2). The ages of the cats varied from 2 months to 15 years old. Of the 471 cats, the sex of 462 cats (232 male, 230 female) was indicated. After collection, all the cat serum samples were treated at 56°C for 30 min to inactivate and stored at −30°C until serological examinations were performed.

Indirect immunofluorescent antibody test for B. henselae

infection: The antibody titers to *B. henselae* were determined by an indirect immunofluorescent antibody test (IFA), using *B. henselae* (ATCC 49882) as an antigen. The strain was cultured on 5% rabbit blood agar plates at 35°C under 5% CO_2 atmosphere for 7–14 days. The cultured organism harvested from the agar plates was suspended in phosphate-buffered saline (PBS, pH 7.4) and washed twice by centrifugation at 12,000 rpm for 10 min. The washed organism was mixed with a cultured cat cell line FCWF (*Felis catus* whole fetus) kindly provided by Dr. Bruno B. Chomel, University of California, Davis, and incubated at 37°C for 24 hr under 5% CO_2 atmosphere in polystylene tissue culture flasks (50-m*l* size). After cultivation, the infected cells were removed with 1 m*l* of a 0.1% trypsin (1:250, Difco, U.S.A.) solution and Dulbecco's modified Eagle medium (Nissui Pharmaceutical Co., Ltd., Japan) supplemented with 10% fetal calf serum was added to make a total volume of 7.5 *ml* of the cell suspension. A volume of 40 μl of the suspension containing the infected cells was dropped onto each well of 12-hole Teflon coated slides (Bocsui Brown, U.S.A.) and cultured for 16 hr at 37°C under 5% CO_2 atmosphere. After incubation, the slides were washed twice in PBS, then fixed in acetone and air-dried. The fixed slides were stored at −20°C until used. A volume of 10 μl of diluted serum was put onto the test holes and the slides were incubated at 37°C for 1 hr in a humid chamber. Then, the slides were washed twice with cold PBS for 15 min. Fluorescein-conjugated goat anti-cat immunoglobulin G (Cappel Products, Organon Teknika Corp., U.S.A.) was diluted 1:800 in PBS with 0.001% Evan's blue and 10 μl of the mixture was applied onto each well. The slides were incubated at 37°C for 1 hr, washed twice with PBS for 15 min, and then washed again with double distilled water for 10 min. The intensity of the bacillus-specific fluorescence was scored subjectively from 1 to 4, and the fluorescence score of $\geqq 2$ at a dilution of 1:64 was considered to be positive. Serum samples were screened at 1:32 and 1:64 dilutions, and any sample positive at 1:64 dilution was titrated in a series of twofold dilutions up to 1:1,024.

Toxoplasma antibody test: The antibody against *T. gondii* was examined with a commercial latex agglutination test kit (Toxo Check; Eiken Chemical Co., Ltd., Japan). A titer of $\geqq$1:64 was considered to be positive.

Table 1. Seroprevalence of *B. henselae* and *T. gondii* in both sexes of pet cats

Sex	Number of cats examined	Number of positives (%) for: *B. henselae*	*T. gondii*
Male	232	30 (12.9)[a)]	21 (9.1)
Female	230	12 (5.2)[a)]	20 (8.7)
Unknown	9	1 (11.1)	0
Total	471	43 (9.1)	41 (8.7)

a) Significantly different between males and females ($p<0.01$).

Detection of FIV antibody and FeLV antigen: Sixty-seven were randomly selected from cat serum samples. Both FIV antibody and FeLV antigen were detected with a commercial assay kit (CITE Combo, IDEXX Laboratories, U.S.A.).

Statistical analysis: The results obtained were analyzed by the χ^2 test.

RESULTS

Of the 471 pet cats examined, 43 (9.1%) were positive for *B. henselae*. Seropositive cats were 30 (12.9%) male, 12 (5.2%) female and one sex-unknown cats (Table 1). The positive rate in male cats was significantly higher than that in female ones ($p<0.01$). In Kanagawa Prefecture, the positive rate varied from 8 (hospital A) to 19.5% (hospital D). In Saitama Prefecture, the rate ranged from 0 (hospital G) to 9.0% (hospital F). The rate was significantly higher in hospital D than that in hospital E ($p<0.05$), but there was no significant difference in the total positive rate between Kanagawa and Saitama Prefectures (Table 2). The ages of the positive cats ranged from 12 months to 14 years and the rate in each age group varied from 4.9 (7 to 10 years) to 12.1% (3 to 4 years) (Table 3).

Forty-one (8.7%) among the 471 cats examined had the antibody to *T. gondii*. The positive rate was 9.1 and 8.7% in male and female cats, respectively. There was no significant difference in the positive rate between male and female cats (Table 1). In Kanagawa Prefecture, the positive rate varied from 4.0 (hospital A) to 18.0% (hospital B). In Saitama Prefecture, the rate was from 5.3 (hospital E) to 18.8% (hospital G) (Table 2). Positive cats were distributed from <1- to 14-year old, but the positive rate tended to be higher in older cats (Table 3). Of the 43 *B. henselae*-positive cats, five (11.6%) were also positive for *T. gondii*, but there was no relationship between *B. henselae* and *T. gondii* seropositivity (Table 4).

Of 67 randomly selected cat serum samples, 16 (23.8%)

Table 2. Seroprevalence of *B. henselae* and *T. gondii* in pet cats in different veterinary hospitals in Kanagawa and Saitama Prefectures

Prefecture	Hospital	Number of cats examined	Number of positives (%) for: *B. henselae*	*T. gondii*
Kanagawa	A	100	8 (8.0)	4 (4.0)
	B	50	5 (10.0)	9 (18.0)
	C	50	6 (12.0)	7 (14.0)
	D	41	8 (19.5)	2 (4.9)
	Subtotal	241	27 (11.2)	22 (9.1)
Saitama	E	114	7 (6.1)	6 (5.3)
	F	100	9 (9.0)	10 (10.0)
	G	16	0	3 (18.8)
	Subtotal	230	16 (7.0)	19 (8.3)
	Total	471	43 (9.1)	41 (8.7)

Table 3. Seroprevalence of *B. henselae* and *T. gondii* in pet cats of different age groups

Age	Number of cats examined	Number of positives (%) for: *B. henselae*	*T. gondii*
<1	81	5 (6.2)	1 (1.2)
1~2>	92	10 (10.9)	6 (6.5)
2~3>	42	5 (11.9)	0
3~4>	33	4 (12.1)	4 (12.1)
4~7>	64	6 (9.4)	3 (4.7)
7~10>	41	2 (4.9)	8 (19.5)
≧10	50	5 (10.0)	11 (22.0)
Unknown	68	6 (8.8)	8 (11.8)
Total	471	43 (9.1)	41 (8.7)

and six (8.9%) were positive for FIV antibody and FeLV antigen, respectively. Among the 16 *B. henselae*-positive cats, four (25.0%) were positive for FIV antibody and one (6.3%) for FeLV antigen. There was no relationship between *B. henselae*-antibody-positive rate and FIV- and FeLV- positive rates (Table 4).

Table 4. Prevalence of *B. henselae*, FIV and FeLV infections in randomly selected 67 pet cats

B. henselae antibody	Number of cats examined	Number of positives (%) for: FIV	FeLV
Positive	16	4 (25.0)	1 (6.3)
Negative	51	12 (23.5)	5 (9.8)
Total	67	16 (23.8)	6 (8.9)

DISCUSSION

The seropositive rate of *B. henselae* is variable depending upon the source of cats and countries examined. In the United States, it was shown that the positive rate varied from 4% in pet cats to 100% in feral cats and the seroprevalence of stray and feral cats was higher than that of pet cats [2, 4, 5, 15, 18, 19]. This suggests that feral and stray cats are more likely to come into contact with infected cats than are pet cats. The positive rate in male cats was significantly higher than that of female ones. Other studies also showed that seropositivity of male cats was higher than that of female ones [4, 27]. These data may suggest that male cats have more chances to be infected with the agent by fights during the estrus period.

In other countries, the seroprevalence was reported to be 8.3% in Switzerland [13], 33.3% in Austria [1], 36% in France [6], and 39.5% in Israel [2]. In Japan, the average seropositive rate to *B. henselae* in cats was reported to be 15.1% [27]. This study revealed that 9.1% of pet cats were seropositive for *B. henselae*. Although all the animals examined were pet cats and most of them except several submitted to the study were clinically healthy, the positive rate to *B. henselae* in both Kanagawa and Saitama Prefectures was rather low compared with those reports mentioned above.

In Japan, a significant difference in the seroprevalence was observed between the northeastern (6.3%, 3/48) and the central areas (22.0%, 13/59) [27]. In the United States, warm, humid environments are associated with a higher seropositive rate than are cold, dry environments [17]. High seropositivity in warm and humid areas may correlate with the existence of many potential arthropod vectors such as fleas. In fact, a number of CSD patients kept kittens infested with cat fleas (*Ctenocephalides felis*), and *B. henselae* and its DNA from fleas were found in carrier cats [18]. Furthermore, transmission of *B. henselae* by cat fleas was experimentally demonstrated in cats [7]. The climate conditions are almost the same in Kanagawa and Saitama Prefectures, and the infestation status of cats with fleas seems to be the same in both prefectures. Therefore, the seroprevalence to *B. henselae* in cats in the Kanto District may depend upon the local hygienic conditions of pet cats rather than the climate.

Several studies found that seropositivity to *B. henselae* was higher in young cats especially under 12 months old than in older ones [4, 5, 9, 18]. On the other hand, Childs *et al*. showed no relationship between the seropositivity and cat age [4]. In this study, the seropositivity to *B. henselae* was not associated with cat age. This suggests that there may be few chances for cats to be infected with *B. henselae* during the kitten period because of the low prevalence of the agent among pet cat population in the Kanto District.

As for the prevalence of *T. gondii* in cats in Japan, the positive rate varied from 10.5% in 1993 to 73.0% in 1972 [12, 14, 16, 17, 22]. In this study, 8.7% (41/471) of pet cats had the antibody against the agent. These data suggest that *T. gondii* infection rate has recently decreased in domestic cats in Japan. The seropositive rate increased with age of cats in the same way as previous reports [12, 22].

Childs *et al*. [4] found that the seropositivity for *B. henselae* was associated with that of *T. gondii*. In this study, only three (7.5%) of 45 cats having *B. henselae* antibody were positive for *T. gondii*, so there was no relationship between *B. henselae* and *T. gondii* seropositivities.

Several reports showed that seropositivity for *B. henselae* was not associated with FIV antibody positivity [5, 13] though the complication of *B. henselae* and FIV infections might be associated with gingivitis and lymphadenopathy in cats [28]. In this study, 67 were randomly selected from the cats examined for FIV antibody and FeLV antigenemia. Among the cats examined, 16 and six were positive for FIV and FeLV, respectively, but there was no relationship between *B. henselae* seropositivity and these viral infections.

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