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Serological Investigation of *Bartonella henselae* Infections in Clinically Cat-Scratch Disease-Suspected Patients, Patients with Cardiovascular Diseases, and Healthy Veterinary Students in Japan

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***Abstract*: Seroprevalence of *Bartonella henselae* was investigated in Japan in 48 individuals clinically suspected of having cat-scratch disease (CSD), 159 patients with cardiovascular diseases, and 129 healthy veterinary students. Of 48 CSD-suspected patients examined, 19 (39.6%) were positive for *B. henselae*-IgG and 4 (8.3%) for *B. henselae*-IgM. Of 159 patients with cardiovascular diseases, 5 (3.1%) were positive for *B. henselae*-IgG. In healthy veterinary students, 14 of 129 (10.9%) were positive for *B. henselae*-IgG and 1 (0.8%) for *B. henselae*-IgM. The positive rates of *B. henselae*-IgG and -IgM in CSD-suspected patients were significantly higher than in other sources. Most CSD-suspected and healthy individuals who were positive for *B. henselae* antibody had had some contacts with cats. In CSD-suspected patients, the *B. henselae* positive rate in females was significantly higher than in males, and high seropositive rates to *B. henselae* were found in younger age groups.**

***Key words*: *Bartonella henselae*, Cardiovascular, Cat-scratch disease, Seroprevalence**

Cat-scratch disease (CSD) is a worldwide zoonosis (9, 14), and cats are regarded as major reservoirs of the disease (1, 2, 9, 10, 18). The Gram-negative, fastidious bacterium, *Bartonella henselae*, has now been recognized as a major causative agent of CSD (9, 14, 18).

Many studies have demonstrated the role of cats in the transmission of *B. henselae* of CSD (2, 8, 9, 18). The number of pet cats is increasing in developed countries and is now suspected of totaling 8.5 million in Japan. According to the increase in the number of pet cats, the problem of zoonoses such as CSD has risen in human society. However, a few seroepidemiological data of CSD in humans have been available because serological diagnosis is available in a limited number of institutions in Japan (15, 16).

In this paper, the authors have investigated the serological prevalence of *B. henselae* infections among CSD-suspected patients, patients with cardiovascular diseases, and healthy veterinary students in Japan.

Materials and Methods

Serum samples. A total of 336 serum samples were collected from October 1995 to October 1999. These samples were from 48 clinically CSD-suspected patients, 159 patients with cardiovascular disease, and 129 healthy veterinary students. Most CSD-suspected patients showed clinical pyrexia and persistent regional lymphadenopathy. The ages of CSD-suspected patients varied from 0 to 73 years (17.5±15.0 yrs.; Mean±S.D.). The ages of patients with cardiovascular disease were from 0 to 94 years (56.0±21.9 yrs.; Mean±S.D.). The samples of healthy veterinary students were collected from 3rd to 6th year students, varying in age from 20 to 43 years (21.8±3.3 yrs.; Mean±S.D.). In this study, cat contacts including scratch and bite, and the history of

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Abbreviations: CSD, cat-scratch disease; IFA, indirect fluorescence antibody.

raising cats and flea infestation were investigated through questionnaires submitted to healthy veterinary students, and these data were also obtained from CSD-suspected patients as much as possible. The serum samples were sent to the Laboratory of Veterinary Public Health, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University in a frozen condition and stored at −80 C until examined.

Indirect immunofluorescence antibody (IFA) test for B. henselae *infection*. The antibody of IgG and IgM for *B. henselae* was determined by IFA test with the antigen of *B. henselae* (ATCC 49882^T). The antigen slides were made by the same procedure reported previously (10). The frozen sera were thawed at room temperature and treated at 56 C for 30 min to inactivate the complement. The IFA procedure was followed by the method reported previously (11). The intensity of the bacillus-specific fluorescence was scored subjectively from 1 to 4, and the fluorescence score of ≧2 was considered to be positive at a dilution of ≧1:64 for IgG and ≧1:16 for IgM antibody.

Statistical analysis. The results obtained from the IFA test was analyzed by the χ^2 test.

Results

The positive rate for *B. henselae*-IgG was 39.6% (19/48) in clinically CSD-suspected patients, 3.1% (5/159) in patients with cardiovascular diseases, and 10.9% (14/129) in veterinary students. For *B. henselae*-IgM, the positive rate was 8.3% (4/48) in CSD-suspected patients and 0.8% (1/129) in veterinary students. No positive case for *B. henselae*-IgM was detected in patients with cardiovascular diseases (Table 1). There were overlapped seropositive cases for *B. henselae*-IgG and -IgM: 2 in CSD-suspected patients and 1 in veterinary students. The positive rates of IgG and IgM antibodies to *B. henselae* in CSD-suspected patients were signifi-

Table 1. Seropositivity against *B. henselae* in 3 human sources

Source	No. examined	No. (%) positive for:	
		B. henselae IgG	*B. henselae* IgM
CSD-suspected patients	48	19 (39.6)*	4 (8.3)**
Patients with cardiovascular diseases	159	5 (3.1)	0
Veterinary students	129	14 (10.9)	1 (0.8)

*The rate is significantly higher than that of other sources ($P<0.001$).
**The rate is significantly higher than that of other sources ($P<0.01$).

Table 2. Seropositivity against *B. henselae* and history of cat contacts and flea infestation in veterinary students

History of:		No. examined	No. (%) positive for: *B. henselae*
Cat contact[a)]	+	111	14 (12.6)
	−	18	0
Flea infestation	+	44	6 (13.6)
	−	80	7 (8.75)
	unknown	5	1 (20.0)

[a)] Cat scratch and/or bite.
+: positive, −: negative.

Table 3. Seroprevalence of *B. henselae* in both genders in 3 sources

Source	Sex	No. examined	No. (%) positive for: *B. henselae*
CSD-suspected patients	Male	25	7 (28.0)
	Female	23	14 (60.9)*
Patients with cardiovascular diseases	Male	101	5 (5.0)
	Female	58	0
Veterinary students	Male	48	2 (4.2)
	Female	81	12 (14.8)

*Significantly different between males and females ($P<0.05$).

cantly higher than those in other groups (IgG; $P<0.001$, IgM; $P<0.01$) (Table 1). Among 19 *B. henselae*-seropositive CSD-suspected patients, 14 (73.7%) had some relations with cats, and 14 (73.7%) have been cat owners. Of 14 *B. henselae* seropositive veterinary students, 10 (71.4%) had been cat owners and 6 are present cat owners (data not shown). The seropositive rate against *B. henselae* in veterinary students who had had contacts with cats or flea bites was higher than in the students who had had none of either (Table 2). *B. henselae* seropositive rate in CSD-suspected patients was 28.0% (7/25) in males and 60.9% (14/23) in females (Table 3). The seropositive rate in females was significantly higher than in males ($P<0.05$). The ages of *B. henselae* seropositive individuals ranged from <10 years to the 70s in three sources. The positive rate of *B. henselae* in younger individuals, such as 18.8% (9/48) in teenagers and 12.5% (6/48) in children less than 10 years old, was more likely to be higher in CSD-suspected patients (data not shown).

Discussion

Clinical symptoms of CSD are similar to those of trench fever, Q fever, atypical mycobacteriosis, tuberculosis, brucellosis, tularemia, infectious mononuclear disease, and Hodgkin's disease. Therefore a serological examination of CSD with IFA is necessary for differential diagnosis of those diseases. And because the cultivation of *B. henselae* is time consuming, the IFA method for the detection antibodies of IgG and IgM for *B. henselae* is useful.

In this study, the *B. henselae*-IgG positive rate in CSD-suspected patients was found to be 39.6%. The positive rates to the organism in CSD individuals varied from 6.3% (26/412) in Italy (4) to 100% (20/20) in Switzerland (12). A higher seropositive rate against *B. henselae* in CSD-suspected patients in Japan indicated that *B. henselae* was also a major causative agent of the disease.

The *B. henselae*-IgG positive rate in healthy veterinary students was found to be 10.9%. In other reports, the positive rate of healthy individuals was reported to be 6.4% in Japan (15), 6.0% and 3.6% in the United States (14, 18), 5.5% in Thailand (11), and 3.0% in Italy (5). On the other hand, the seropositive rate in veterinarians was reported to be 8.1% (13). Cats may infect humans directly through scratches, bites, or licks; or indirectly via an arthropod vector (18). In our study, 73.7% (14/19) of *B. henselae* seropositive CSD-suspected patients and 100% (14/14) healthy veterinary students had some contacts with cats. These data suggested that cat contacts, including scratches and bites, might be significant risk factors for CSD.

It was recently reported that *B. henselae* caused endocarditis in immunocompetent individuals (6); however, the seropositive rate against *B. henselae* in patients with cardiovascular diseases was 3.1%, indicating a similarity in healthy individuals. It seems very unlikely that *B. henselae* is a major causative agent of cardiovascular disease in Japan.

The positive rate of *B. henselae*-IgM in CSD-suspected patients was 8.3%. This rate was much lower than the rates reported in other studies in Japan (24.2%, 20.0%) (16, 17) and in Switzerland (90.0%) (19). These differences may be attributed to the difference of times when serum samples were collected.

A high seroprevalence of *B. henselae* in warm areas was reported, and arthropod vectors including fleas might be associated with the infections in cats (8). Furthermore, *B. henselae* was isolated from fleas (*Ctenocephalides felis*) infested in 9 carrier cats, and the organism was experimentally transmitted among cats by fleas (3, 9). In our study, the seropositive rate against *B. henselae* in healthy veterinary students with flea contacts was not significantly different from those without flea contacts. Therefore fleas seemed to play quite limited roles in the transmission of the organism in humans.

Although it was reported that a *B. henselae* positive rate in males was higher than in females (1, 7), our data showed that the rate of CSD-suspected patients was significantly higher in females than that in males ($P<0.05$). This may suggest that females in Japan have more interest in cats and therefore more chances to come into contact with cats than males do.

A high seropositive rate against *B. henselae* was shown at the age of 10 than in older generations (1, 7). In our study, higher seropositivity was also found in the age of 10s and under 10 years old in comparison with older CSD-suspected patients. Younger people are playful and more likely to have contact with cats. Therefore they may have more opportunities to be scratched or bitten by cats than people 20 and older.

Our serological data suggest that cats play a significant role as a reservoir of CSD in Japan because most individuals seropositive to *B. henselae* had some relationship with cats. Furthermore, it is of interest that higher positive rates to *B. henselae* were found in females and younger age groups. Further study will be necessary to clarify the epidemiology of CSD in Japan.

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