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The seroprevalence of *Bartonella henselae* in healthy adults in Korea

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Background/Aims: Cat-scratch disease (CSD), caused by *Bartonella henselae* is one of the most common zoonosis. However, only several cases of *B. henselae* infection have been reported in Korea. This study investigated the seroprevalence of *B. henselae* in healthy adults and related risk factors.

Methods: Serum samples from 300 healthy participants were analyzed using an immunoglobulin G immunofluorescence assay (IFA) for *B. henselae* isolated in Korea. Surveys on the risk factors for *B. henselae* infection were conducted simultaneously.

Results: Of the participants, 47.7% and 15.0% raised dogs and cats, respectively. The overall seroprevalence of *B. henselae* was 15.0% (IFA titer ≥ 1:64). Participants who had raised cats showed 22.2% seropositivity against *B. henselae*, and those with no experience with cats showed 13.7% seroprevalence (p = 0.17). Participants who had cats as pets or been scratched by cats, showed 9.8% seropositivity against *B. henselae* (IFA titer ≥ 1:256). However, those who had not raised or been scratched by a cat showed 2.0% seropositivity (p = 0.015).

Conclusions: In Korea, the seroprevalence of *B. henselae* is higher than expected, suggesting that *Bartonella* infection due to *B. henselae* is not uncommon. Cats are proposed to play a more important role than dogs in transmission of CSD.

Keywords: Bartonella henselae; Seroepidemiologic studies; Korea; Cat-scratch disease

INTRODUCTION

Bartonella species have recently been proven to cause zoonosis. *Bartonella* spp. are gram-negative, intracellular bacterial pathogens, and 20 named species belong to the genus [1]. Among them, *Bartonella henselae, Bartonella bacilliformis,* and *Bartonella quintana* are important pathogens that cause infections in humans [1]. *B. henselae* is commonly transmitted when humans are scratched or bitten by cats or dogs [2-4]. Once infected, most people present with various clinical symptoms, including cat-scratch disease (CSD), trench fever, infective endocarditis, bacillary angiomatosis, lymphadenopathy, fever of infective endocarditis, fever of unknown origin, myalgia, neuropathy, and uveitis [5,6].

In the United States, CSD caused by *B. henselae* is one of the most common zoonosis. The disease results in more than 25,000 patients per year [7]. Moreover, the seropositivity rates against *B. henselae* among healthy adults are 16.0% in Sweden, 8.7% in Spain, and 19.6% in China [8-10].

In Korea, only a few cases of *Bartonella* infection have been reported [2]. A 14-year-old boy was reported as having cervical lymphadenopathy caused by *Bartonella*

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infection; this was the first proven case diagnosed with polymerase chain reaction (PCR) [11]. According to the Seoul Institute, 640,000 households (16.7%) of Seoul citizens have pets [12]. Approximately, 16% to 43% of specimens (i.e., blood, nail, and saliva) from dogs and cats were positive for *B. henselae*, as confirmed by PCR [3]. Chae et al. [13] reported that 38.7% of patients with lymphadenopathy showed high titers of *B. henselae* immunoglobulin G (IgG) (≥ 1:128).

These prior studies suggest that a higher incidence of *B. henselae* infection should be expected. The incidence of *B. henselae* infection and risk factors associated with CSD should be recognized in Korea, because many physicians are not aware of this disease. In this study, we investigated the seroprevalence of *B. henselae* in healthy Korean adults and related risk factors.

METHODS

Study population

Ethical approval was granted by the Institutional Review Board of Inha University Hospital (IUH-IRB 13-2793). Serum samples were obtained from study participants who visited Inha University Hospital in Incheon, Korea, for a health examination from January to December 2014. All participants provided informed consent.

Questionnaire content

The participants were asked to complete a questionnaire providing personal and epidemiological information on age, sex, living and working environment, history of contact with pets, experience of being scratched by pets, history of admission because of fever or lymphadenopathy, history of uveitis, level of physical activity, and history of travel abroad. All questionnaires were distributed by the two interviewers who were trained in advance regarding the interview contents.

Serological testing and cultivation of *Bartonella* species

ECV304 cells infected with a strain of *B. henselae*, which was previously isolated from a clinical specimen (Genebank registration No.JQ638927.1) were grown and subjected to an in-house immunofluorescence assay (IFA) analysis [14]. The infected cells were smeared on the spot slides and fixed using acetone for 10 minutes at 4°C. Sera from healthy participants were serially diluted from 1:40 to 1:1,280 and incubated with the pre-prepared slides at 37°C for 30 minutes. After washing three times with phosphate buffered saline, fluorescein-labeled goat anti-human IgG antisera was applied and the slides were incubated under the same conditions for the primary antibody reaction. Slides were mounted with anti-fluorescence media (Vector, Burlingame, CA, USA) and observed using a fluorescence microscope (Axioskop 2, CarlZeiss, Gottingen, Germany) at ×200 magnification. The sera were simultaneously examined with commercially available slides for *Bartonella*-IFA IgG assay (Focus Diagnostics, Cypress, CA, USA), according to the inserts provided by the manufacturer.

The laboratory results based on the in-house IFA analysis were presented and two cutoff points for seropositivity were used: 1:64 and 1:256; 1:64 is the documented cut-off value, and 1:256 was used to include the proportion of participants who are more likely to have been infected or with presumptive evidence of recent infection with *B. henselae*.

Statistical methods

Data were analyzed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Univariate analysis (chi-square test and *t* test) was performed to determine the impact of different independent risk factors.

RESULTS

Among 300 healthy participants, 113 (37.7%) were men and 187 (62.3%) were women. Sixty participants (20%) were enrolled in each age group (i.e., 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 years and older).

Corresponding questionnaires were completed by the 300 participants (Table 1). Of these, 143 (47.7%) reported that they were raising or had raised dogs, 64 (15.0%) had been scratched by dogs, 25 (8.3%) were raising or had raised cats, and 39 (13.0%) had tended animals other than dogs or cats. Additionally, five subjects (1.7%) had been hospitalized because of febrile disease, and two patients had a history of lymphadenopathy. Forty-five of the participants (15%) were seropositive for *B. henselae* IgG (1:64 or higher). In addition, among the seropositive

Table 1. Questionnaire answers of the subjects (n = 300)

Variable	No. of participants (%)
Experience of raising dogs	143 (47.7)
Experience of being scratched by dogs	64 (15.0)
Experience of raising cats	25 (8.3)
Experience of tending other animal	39 (13.0)
Hospitalization because of febrile illness	5 (1.7)
Experience of being scratched by other animals	2 (0.7)

Table 2. Age groups of the subjects with *Bartonella henselae* seropositivity of 1:64 or higher

Age group, yr	Seronegative	Seropositive	Number
20–29	55 (91.6)	5 (8.3)	60
30–39	49 (81.6)	11 (18.3)	60
40–49	54 (90.0)	6 (10.0)	60
50–59	50 (83.3)	10 (16.6)	60
> 60	47 (78.3)	13 (21.6)	60
Total	255	45	300

Values are presented as number (%).

individuals, the proportion of men (51.1%) was slightly higher than that of women (48.9%); however, the difference was not statistically significant (p = 0.44). Each age group showed varying seropositivity, ranging from 8.3% to 21.7%; 8.3% for the 20 to 29 age-group, 18.3% for the 30 to 39, 10.0% for the 40 to 49, 16.6% for the 50 to 59, and 21.6% for the group aged 60 years or above. Individuals aged older than 60 years had the highest rate of seroprevalence, but this difference was not statistically significant (p = 0.24) (Table 2, Fig. 1).

The seroprevalence of *B. henselae* (IgG titer of 1:64 or higher) in this study was 15.0% (45/300) (Table 3). The individuals who were *B. henselae* seropositive (IgG titer of 1:64) were more likely to have raised or been scratched by cats. However, the results were not statistically significant.

Ten of the participants (3.3%) had *B. henselae* IgG titers of 1:256 or higher (Table 4); five (3.5%) were among the 143 individuals who had raised dogs and five (3.2%) were among the 157 participants who had not raised dogs. *B. henselae* seroprevalence of 8.9% (4/45) was observed among the participants who had raised cats and 2.4% (6/255) among those who had a history of raising cats, and this result was statistically significant (p = 0.05). Among those who had a history of raising cats and being scratched by cats, 9.8% (5/51) were seropositive for *B. henselae*. Among those who did not have a history of raising cats and having been scratched by cats, 2.0% (5/249) were seropositive, and this result was statistically significant (p = 0.02).

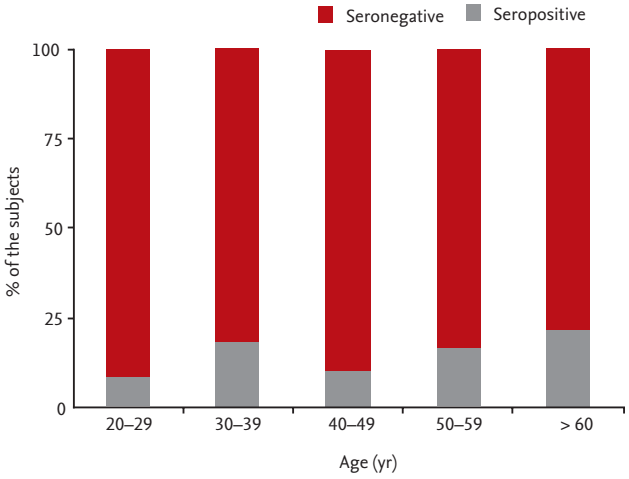

Figure 1. Graph by age grouping of the participants with *Bartonella henselae* immunoglobulin G titers of 1:64 or higher.

However, a history of raising dogs or a history of raising and being scratched by dogs was not significantly associated with higher titers (i.e., 1:256 or higher) (p = 0.57

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Table 3. *Bartonella henselae* seroprevalence of 1:64 or higher according to risk factors

Risk factor	Seropositive subject		*p* value
	With risk factor	Without risk factor	
Have raised dogs	23/143 (16.1)	22/157 (14.0)	0.63
Have been scratched by dogs	9/64 (14.1)	36/236 (15.3)	1.00
Have raised cats	10/45 (22.2)	35/255 (13.7)	0.17
Have been scratched by cats	5/25 (20.0)	40/275 (14.5)	0.31
Have raised dogs and have been scratched by dogs	25/160 (15.6)	20/140 (14.3)	0.87
Have raised cats and have been scratched by cats	11/51 (21.6)	34/249 (13.7)	0.19
Have raised dogs and cats	25/148 (16.9)	20/152 (13.2)	0.42
Have raised and have been scratched by dogs or cats	27/168 (16.1)	18/132 (13.6)	0.63

Values are presented as number (%).

Table 4. *Bartonella henselae* seroprevalence of 1:256 or higher according to the risk factors

Risk factor	Seropositive subject		*p* value
	With risk factor	Without risk factor	
Have raised dogs	5/143 (3.5)	5/157 (3.2)	0.57
Have been scratched by dogs	0/64 (0.0)	10/236 (4.2)	0.09
Have raised cats	4/45 (8.9)	6/255 (2.4)	0.05[a]
Have been scratched by cats	2/25 (8.0)	8/275 (2.9)	0.20
Have raised dogs and have been scratched by dogs	5/160 (3.1)	5/140 (3.6)	0.54
Have raised cats and have been scratched by cats	5/51 (9.8)	5/249 (2.0)	0.02[a]
Have raised dogs and cats	7/148 (4.7)	3/152 (2.0)	0.16
Have raised and have been scratched by dogs or cats	6/168 (4.2)	3/132 (2.3)	0.28

Values are presented as number (%).
[a]Statistically significant result.

and $p = 0.54$, respectively).

In this experiment, both in-house and commercial IFA slides were used to detect *B. henselae* seropositivity. *B. henselae* isolated from Korea was used as the antigen for in-house slides to improve the accuracy of the detection. The sensitivity of assays using in-house slides compared those using commercial slides was 84.8%, and the specificity was 97.6%, suggesting that the in-house assays are reliable for assessment of *B. henselae* antibody titer (Table 5).

DISCUSSION

Several seroepidemiologic studies in healthy populations have been conducted to investigate *Bartonella* spp. seroprevalence. The seroprevalence of *Bartonella* infection varies by region. A study of blood donors in Sweden showed 16.1% seropositivity for *B. henselae* and *B. quintana* [8]. Seroprevalence of *B. henselae* among healthy adults was reported to be 8.7% in Spain and 19.6% in Zhejiang province in China [9,10].

Only a few cases of *B. henselae* infection have been reported in Korea. However, *B. henselae* DNA has been detected in specimens from the reservoir of animals such as cats and dogs, and higher titers for *B. henselae* were documented in patients with lymphadenopathy [3]. These prior studies indicate a high number of unreported patients with *Bartonella* infection. In the current study, 15.0% of asymptomatic healthy Korean adults were seropositive for *B. henselae* (IgG, 1:64 or higher). In addition, 3.0% of healthy participants had IgG titers of 1:256 or higher, indicating the greater likelihood of a recent infection. *Bartonella*-IgG IFA is known to have cross reactivity with other diseases [15,16]. Even considering that, however, 15.0% seroprevalence suggests that *Bar-*

Table 5. Sensitivity and specificity of IFA results: commercial vs. in-house

Variable	In house IFA	
	Positive	Negative
Commercial IFA		
Positive	39 (84.8)	7 (15.2)
Negative	6 (2.4)	248 (97.6)

Values are presented as number (%).
IFA, immunofluorescence assay.

tonella infection is not uncommon in Korea.

B. henselae infection may present with various nonspecific clinical symptoms, which causes difficulty in the diagnosis of this infection. Also, in pathological examination, the infection demonstrates granulomatous lymphadenitis. Therefore, it may be misdiagnosed as tuberculosis or Kikuchi-Fujimoto disease, which is known to be more common causes of lymphadenitis in Korea [17]. Furthermore, since *B. henselae* is sensitive to antibiotics such as rifampicin, it may improve clinically with the anti-tuberculosis medication. In Korea, the high incidence of Kikuchi disease has been reported as a cause of lymphadenopathy. Physicians should be aware that *B. henselae* infection is also an important cause of lymphadenopathy.

B. henselae infection is a zoonosis, and animals play an important role in its transmission. Cats are known to be the most important transmitter, although other animals including dogs may play a role as well [3]. In a study conducted in Spain, 31.6% of seropositive individuals reported exposure to cats and 5.6% of people reported exposure to dogs [9]. In our study, 143 participants (47.7%) had experience tending dogs, while only 25 (8.3%) had tended cats. Traditionally, Koreans have had a predilection for dogs above cats. Therefore, we expected that dogs may serve as a reservoir for *B. henselae* infection. Chae et al. [13] did not provide evidence of a statistically significant association of *B. henselae* seropositivity with raising animals, including cats. In our study, tending cats or a previous experience of tending cats showed a statistically significant association with *B. henselae* seropositivity. The experience of tending dogs did not show a statistically significant association with *B. henselae* seropositivity. Therefore, our study concludes that cats are more strongly associated with *B. henselae* infection than dogs, which corresponds with previous studies.

Our study showed that the seroprevalence of *B. henselae* among healthy Korean adults is also high and seropositivity is associated with history of tending cats or being scratched by cats. Physicians should be aware of CSD, considering the rate of *B. henselae* seropositivity among the healthy Korean adults in this study was 15.0 %. In addition, further study is needed to determine whether *Bartonella* infection causes clinical symptoms such as lymphadenitis, acute febrile illness, and uveitis in adults.

KEY MESSAGE

1. In Korea, *Bartonella* infection is neglected but common disease. Physicians need to have high suspicion to diagnose the disease.
2. Contact with cats (raising cats, scratch) may play an important role in transmission of *Bartonella henselae*.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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