Isolation of *Bartonella henselae* from Domestic Cats in Japan

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**Abstract.** During the period from January to March 1995, the authors first isolated *Bartonella henselae* from the blood of three (9.1%) of 33 domestic cats in Japan. The three cats were a 1.5-year-old male cat-old with urinary retention, and 6-year-old female pound and age-unknown female cats with no abnormalities. The blood was taken in a lysis-centrifugation tube (Wampole Isolator tube) and cultured on 5% rabbit-blood heart infusion agar plates at 35°C in the 5% CO2 atmosphere. Visible tiny rough colonies developed 14 days after incubation. The isolates showed Gram-negative and pleomorphic rods in microscopic observation. The DNA extracted from the isolates was amplified by PCR using two primers, which were specific for the rikettisal citrate synthase gene. The isolates were identified as *B. henselae* from the patterns of digestion with *Taql* and *Hhal* of the amplified gene. It was confirmed that cats in Japan harbored *B. henselae* in their blood, and that cats play a significant role as the reservoir of the organism. — **Key words: Bartonella henselae, cat scratch disease, feline.**


Cat scratch disease (CSD) in humans is characterized by pyrexia, persistent regional lymphadenopathy, and skin lesions such as papule, small vesicle, and ulcer at the site of a cat scratch or bite [1, 4, 6, 15, 16]. The causative agent of CSD has remained unknown for a long time. The agent of CSD has provisionally been associated with a fastidious Gram-negative rod bacterium, *Afipia felis* [2, 5]. However, *A. felis* has rarely been isolated from patients with CSD, and serological investigation on the patients of CSD showed little participation of *A. felis* in this disease. Recent investigations suggest that *Bartonella henselae*, formerly named *Rochalimaea henselae*, is a causative agent of bacillary angiomatosis (BA) and bacillary peliosis hepatitis in HIV-positive patients [11, 14]. Furthermore, the organism has been isolated from patients of CSD [4] and serological investigations on the patients strongly suggest that the organism is the causative agent of CSD [9].

In Japan, although human clinical cases of CSD have been reported, all the cases were diagnosed from the clinical manifestations, the contact with cats, skin test results [1] and/or detection of Warthin-Starkey-staining positive organisms in the lymphnodes [6, 15]. However, there is no report on the isolation of *B. henselae* from either humans or cats. In this paper, the authors report the isolation of *B. henselae* from three domestic cats in Japan.

During the period from January 20 to March 9, 1995, a total of 24 pet and 9 pound cats were examined for *B. henselae*. For the isolation of the organism, pediatric lysis-centrifugation tube (Wampole Isolator tube; Wampole Labs, Cranbury, NJ, U.S.A.), 5% rabbit-blood heart infusion agar (RBHIA) plates, and supplemented medium 199 were prepared. The supplemented medium 199 comprised 10 ml of (10 ×) M-199 medium (GIBCO, Grand Island, NY, U.S.A.), 1 ml of 200 mM L-glutamine (GIBCO), 1 ml of 100 mM sodium pyruvate (GIBCO), 3 ml of 7.5% sodium bicarbonate (GIBCO), 20 ml of fetal bovine serum (GIBCO), and 65 ml of H2O, with pH adjusted to 7.1 to 7.4 by adding 7.5% sodium bicarbonate (GIBCO). A 1.5-ml volume of blood collected from each cat was put in a Wampole Isolator tube and mixed well. The tube was centrifuged at 3,800 rpm for 70 min. The supernatant was aspirated and the sediment was suspended with 120 μl of the supplemented medium 199. The suspension was inoculated onto two RBHIA plates, which were incubated at 35°C for 3 weeks in the 5% CO2 atmosphere. Visible colonies formed on the agar plates were observed 7, 14 and 21 days after incubation. When suspicious colonies were formed on RBHIA plates, three to five were picked up and subcultured under the same conditions as stated above.

The time required for colony formation, colony morphology and Gram staining of the organisms were compared to the type strain of *B. henselae* (ATCC 49882). The DNA was extracted with an Instagen (Bio-Rad, Hercules, CA, U.S.A.) from each isolate and amplified by polymerase chain reaction (PCR) using two synthetic oligonucleotide primers specific for the rikettisal citrate synthase gene. The species of *Bartonella* was identified by the restriction fragment length polymorphism (RFLP) analysis after digestion of the amplified gene with *Taql* and *Hhal* (Takara, Ohtsu, Shiga, Japan) [8].

The organism was isolated from three (9.1%; cat Nos. 1, 2, and 3) of 33 cats investigated. Cat No. 1 was a 1.5-year-old male pet cat weighing 4.0 kg with urinary retention. Cat No. 2 was a 6-year-old female pound cat weighing 1.8 kg. Cat No. 3 was an age-unknown female pet cat weighing 4.6 kg. Both cats Nos. 2 and 3 seemed to be healthy. Tiny rough colonies resembling those of *B. henselae* type strain were visible on the 14th day and became larger (1-3 mm in diameter) on the 21st day after incubation (Fig. 1). The characteristics such as Gram-negative, pleomorphic (2 × 0.5 μm, Fig. 2), and fastidious in growth were observed in these cats’ isolates and they were similar to those of the type strain. A fragment of the citrate synthase gene (approximately 400 bp) was amplified from the DNA
extracted from the three isolates. Furthermore, the RFLP profile after digestion with TaqI and HhaI revealed the same patterns with that of the type strain of \textit{B. henselae} (Fig. 3). These isolates were identified as \textit{B. henselae}. A serum sample was obtained from one (cat No. 3) of the three cats and examined for the antibody to \textit{B. henselae}, but no positive titer against the organism was found.

In Japan, the suspicious agent of CSD have been detected in histological examinations of clinical specimens of patients [6, 15], but the isolation was not successful. Furthermore, there has been no report on isolation of \textit{B. henselae} from cats in Japan. In the present study, it was found that 9.1\% of the cats investigated were being infected with \textit{B. henselae}. Although the cats examined were suffering from bacteremia...
due to the organism, none of them showed any significant clinical manifestation except for cat No. 1 with urinary retention. It did not seem, however, that the urinary retention was caused by the organism, since the cats harboring the organism in their blood showed no clinical signs [7, 10, 16]. Therefore, it was suggested that B. henselae could cause subclinical infection in cats.

It was shown that both CSD and BA were strongly associated with keeping a kitten and/or being bitten or scratched by a cat or kitten [7, 12, 16]. Koehler et al. reported that 41% of cats in the San Francisco area were harboring B. henselae in their blood and BA infection in humans was directly associated with B. henselae bacteremic cats [7]. In the United States, 77 (13.0%) of 592 cats unrelated to CSD had the antibody to B. henselae [3]. Seropositivity to B. henselae in CSD patients’ cats was higher (79.2%, 38/48) than that in control cats (37.9%, 11/29) [16]. In Japan, the antibody to the organism was found also in 30 (15.1%) of 199 cats, though some geographical difference was shown [13]. These and our present data suggest that cats play a significant role as a reservoir of B. henselae.

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REFERENCES