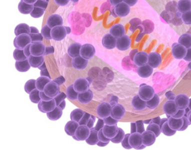

Case Report

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First Case of *Bartonella henselae* Bacteremia in Korea

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Bartonella henselae causes cat-scratch disease, bacteremia, and various focal infections. Despite the worldwide occurrence of *B. henselae* infections, reports in humans are rare in Korea. The clinical manifestation of all 5 previously reported cases was lymphadenopathy. Herein, we report a case of bacteremia in a woman who presented with prolonged fever. *B. henselae* was isolated from a blood specimen by cell culture. Conventional polymerase chain reaction amplification and sequencing of the 16S-23S rRNA intergenic space region confirmed the isolate to be *B. henselae*. The patient had no underlying immunocompromising conditions and no recent exposure to animals. She was successfully managed with a combination of doxycycline and hydroxychloroquine.

Key Words: *Bartonella henselae*, Bacteremia, Fever of unknown origin, Doxycycline, Chloroquine

Introduction

Bartonella species are gram-negative, facultative intracellular bacilli, and the genus includes more than 20 species or subspecies. Among these species and subspecies, *Bartonella henselae* is the primary species that causes human illnesses, including lymphadenopathy, bacteremia, bacillary angiomatosis, and other localized infections. *B. henselae* infection is associated with exposure to cats and possibly dogs. Cat fleas are thought to be the main vector of *B. henselae*. Cat bites and the scratching of flea-bite sites are thought to be the main routes of transmission to humans and cats.

There have been several reports of *Bartonella* infection in Korea. In one study, among 31 patients with cervical lymphadenopathy, 21 (67.7%) and 20 (64.5%) patients had positive serologic results for *B. henselae* and *B. quintana*, respectively [1]. Using polymerase chain reaction (PCR) analysis, the prevalence of *Bartonella* infection was found to be 0–44.1% in animals [2-5] and 0–19.1% in arthropod vectors [3, 5, 6]. Considering that the rates of *Bartonella* spp. infection in both cats and cat fleas are high in Korea [3, 5, 6], it is rather surprising that there have been only 5 case reports of cat-scratch disease [7, 8] and 1 case report of *B. quintana* endocarditis [9] published. All these previously reported cases were diagnosed using PCR. To our knowledge, no case of culture-proven *Bartonella* bacteremia has been reported thus far in Korea.

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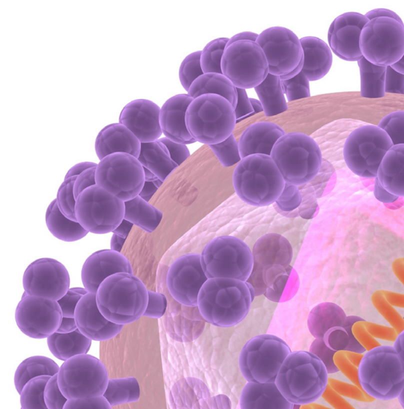

There are many clinical manifestations of *Bartonella* infection. Bacteremia has been known to occur, typically in immunocompromised patients [10]. However, with advanced diagnostic methods, such as the use of a novel growth medium [11], *B. henselae* bacteremia is increasingly identified in immunocompetent patients [12]. We report a case of culture-proven *B. henselae* bacteremia in a patient presenting with fever of unknown origin (FUO).

Case Report

A 73-year-old woman was admitted to our hospital for evaluation of fever. The patient had diabetes mellitus that had been managed with oral hypoglycemic agents for 10 years. Six years previously, the patient underwent an orthopedic operation involving the insertion of internal fixation due to a right ankle fracture. She had no recent history of outdoor activities or contact with pet animals. Two weeks before admission, she developed fever without any localized symptoms. One week later, the patient visited the emergency room of our hospital for evaluation of fever. At that time, her body temperature was 39°C. To identify the cause of the fever, laboratory tests were conducted, which revealed the following: hemoglobin level, 12.9 g/dL; white blood cell (WBC) count, 5,120/mm^3; platelet count, 164,000/mm^3; aspartate aminotransferase level, 47 IU/L; alanine aminotransferase level, 56 IU/L; alkaline phosphatase level, 228 IU/L; C-reactive protein (CRP) level, 131.6 mg/L; and erythrocyte sedimentation rate, 22 mm/h. Blood culture grew coagulase-negative staphylococcus in 1 of the 2 sets, which was dismissed as a contaminant. An abdomino-pelvic computed tomography scan revealed neither splenomegaly nor lymphadenopathies. The patient refused to be admitted to hospital; therefore, oral amoxicillin-clavulanate was prescribed. The patient was scheduled to return to the outpatient department (OPD). The patient's condition did not improve while she was at home. Thus, the patient was admitted to our division on May 22, 2009.

On admission, she had a body temperature of 39.0°C, a pulse rate of 90/min, a respiration rate of 18/min, and blood pressure of 120/80 mmHg. Physical examination revealed no evidence of rash, eschar, or lymphadenopathy. Her WBC count had increased to 9,930/mm^3, her platelet count to 189,000/mm^3, and her CRP level to 179.2 mg/L. Other laboratory results, including repeated blood culture, revealed no significant changes. To determine the cause of the headache, cerebrospinal fluid was examined, which showed no abnormal findings. Gallium and positron emission tomography scans revealed no significant abnormalities. Transesophageal echocardiography revealed mild aortic regurgitation and no vegetation on the valve. Ceftriaxone was administered empirically for 5 days, followed by a combination of ceftriaxone and doxycycline for 6 days. Administration of these antibiotics resulted in minimal clinical improvement. Owing to her persistent fever, vancomycin and doxycycline were substituted on hospital day (HD) 16, and as a result, her fever decreased to 37.5°C the next day. On HD 23, serologic test results for Q fever, which were analyzed at the Korea Centers for Disease Control and Prevention, were positive at titers of 1:64 for immunoglobulin (Ig) M and 1:1,024 for IgG in a blood specimen taken on HD 7.

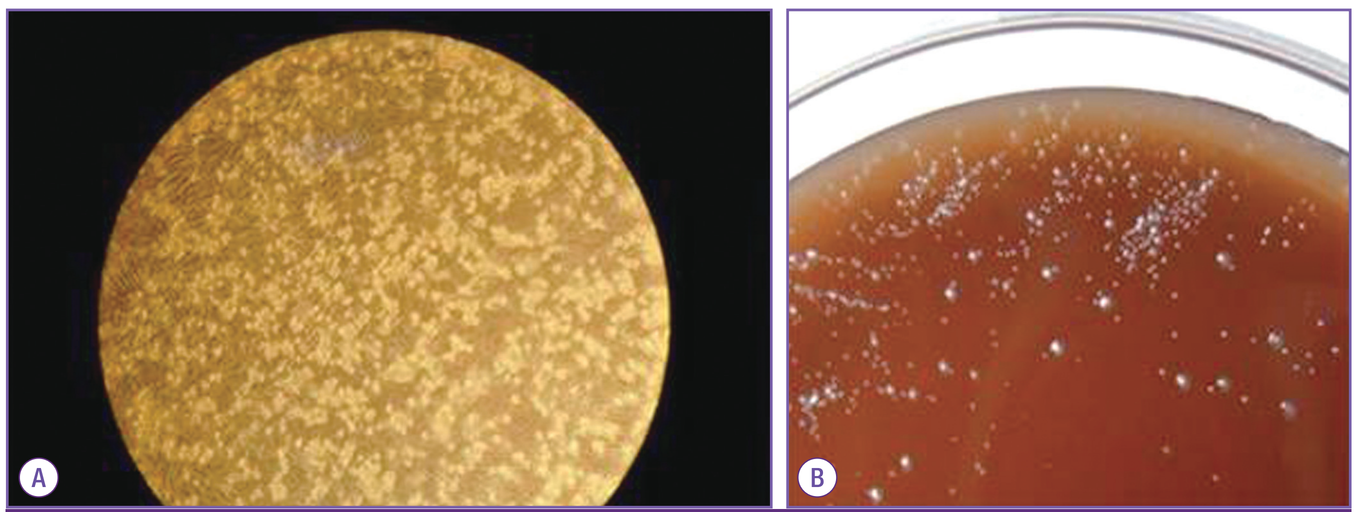

Figure 1. (A) Cell culture using ECV304 cells, 3 months after inoculation of the patient's blood specimen, revealed clusters of round refractile cells (×100 magnification); (B) Culture on blood agar showed small transparent colonies 9 days after inoculation.

The treatment regimen was changed to a combination of doxycycline and hydroxychloroquine to target the possible chronic Q fever, and the fever reduced to below 37°C. The patient was discharged on HD 29, and followed up at the OPD on July 3, 2009. At this follow-up visit, antibody titers to Q fever were 1:32 and 1:2,048 for IgM and IgG, respectively.

We performed cell culture or shell vial culture assays routinely for all patients with FUO. EDTA-treated whole blood (0.3 mL) obtained at admission was inoculated onto a monolayer of ECV304 cells grown in a tissue culture flask (25 cm^2). The inoculated blood was washed with phosphate buffered saline 24 h later, and the culture was maintained in M199 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL) in a humidified atmosphere containing 5% CO_2 at 37°C. Media were changed every 3 to 4 days without subculture. Three months after inoculation, certain cytopathic effects were observed (Fig. 1A). Nine days after subculture onto a blood agar plate, small transparent colonies were observed (Fig. 1B). To identify the isolated bacterium, conventional PCR targeting the 16S-23S rRNA intergenic spacer (ITS) region of *Bartonella* species was performed, followed by sequencing, as described previously [13]. The pair of primers used for the amplification of the ITS gene were 16SF (5′-AGAGGCAGGCAACCACGGTA-3′) and 23SI (5′-GCCAAGGCATCCACC-3′). The sequence alignment of the PCR product was 960 characters long (GenBank accession number: JQ638927.1). Pair-wise comparison using BLAST (National Institutes of Health, Bethesda, MD, USA) revealed that the isolate was most like *B. henselae* Houston-1 strain (maximum pairwise identity score: 98%).

Discussion

We have presented the first case of culture-proven *Bartonella* bacteremia in Korea. *Bartonella* species are facultative intracellular gram-negative bacilli. Therefore, conventional blood culture cannot cultivate the bacterium. Instead, cell culture, direct inoculation of blood specimens onto agar plates, or the use of *Bartonella* alpha *Proteobacteria* growth medium must be used to successfully cultivate the bacterium. However, these culture techniques are seldom used in Korean hospitals, and hence, isolation of the bacterium has never been reported. There are advantages associated with the use of culture methods. If isolated, the pathogen can then be used for further study. The isolate described above was used for the production of monoclonal antibodies [14]. In addition, one cause of false-negative results obtained using an indirect immunofluorescent assay (IFA) is thought to be the variation in antigenic expression between *B. henselae* strains. IFA is thought to be more sensitive if a locally isolated strain is incorporated into the panel of strains used as antigens. PCR is more convenient than the culture method. Therefore, PCR has been used for patients with clinically suspected *Bartonella* infection, who usually exhibit lymphadenopathy or have a history of exposure to cats or dogs. The 5 cases of *B. henselae* lymphadenitis documented in Korea were diagnosed in this manner. Serologic tests have low sensitivity, yield inconsistent results in some patients, and are subject to significant cross-reactivity with other pathogens. For example, in the present case, the seropositivity for Q fever was thought to be due to cross-reaction with *Bartonella* species antigens. Although co-infection with *Coxiella burnetii* and *B. henselae* cannot be excluded, our previous experience with a scrub typhus case found a false-positive result for *C. burnetii* [15]. In addition, a report revealing occasional false-positive results for *C. burnetii* in bartonellosis [16] leads us to disregard the positive serologic results for *C. burnetii*. The drawbacks of serologic testing had rendered them unavailable in most Korean hospitals and referral centers, with the exception of the Korea Centers for Disease Control and Prevention. Pathologic examination using silver staining methods has been used to diagnose *Bartonella* lymphadenopathy, but is not useful in the diagnosis of bacteremia.

Although bartonellosis is an important cause of FUO [17], previous studies of FUO in Korea have not evaluated bartonellosis. Therefore, the level of clinical suspicion of bartonellosis in patients with FUO is lower than that in patients with lymphadenopathy. In addition, clues suggesting bartonellosis, such as contact with cats or insects, are often lacking, which makes diagnosis more difficult. Furthermore, *Bartonella* infection in elderly persons frequently presents with atypical manifestations and is not accompanied by lymphadenopathy; thus, this diagnosis is often disregarded. Screening by laboratory tests is necessary for diagnosing bartonellosis in patients with prolonged fever, even in those lacking the characteristics usually associated with bartonellosis.

The mode of transmission of *Bartonella* in our patient is unclear. Exposure to cats or possibly dogs is a risk factor, but many patients with bartonellosis do not report exposure to pet animals. Instead, because *Bartonella* DNA has been detected in various arthropod vectors including ticks and mites, patients may become infected via these vectors. Given that *B. henselae* can cause persistent infection, recrudescence may be an alternative explanation, though the patient did not have a history

of bartonellosis.

The patient in the present report was successfully managed with a combination of doxycycline and chloroquine, although this treatment's efficacy against *B. henselae* bacteremia is unproven. Currently, there is no standard treatment for *B. henselae* bacteremia. A combination of doxycycline and gentamicin is recommended for *Bartonella* endocarditis [18], and azithromycin and rifampin in combination have been used in some patients with *B. henselae* bacteremia. Although the effects of chloroquine in the treatment of bartonellosis are currently not known, its effect has been established in other intracellular bacterial infections such as Q fever and Whipple's disease. In these diseases, chloroquine enhances the antimicrobial efficacy of doxycycline through the alkalization of acid vesicles such as phagosomes [19]. Although *B. henselae* does not proliferate in acidic vesicles [20], in the present case, the addition of chloroquine to doxycycline seemed to be more efficacious in controlling the fever than doxycycline alone (ceftriaxone and doxycycline, vancomycin and doxycycline). Therefore, chloroquine may potentiate the antibiotic effect in *Bartonella* infection via a mechanism other than alkalization of phagosomes. Further evaluation of the efficacy of adding chloroquine to doxycycline is needed.

In summary, we have reported a case of culture-proven *B. henselae* bacteremia in a patient who presented with FUO. This case suggests that bartonellosis should be included in the list of differential diagnoses in FUO, even in patients lacking the common characteristics of cat-scratch disease. Because the characteristic findings of bartonellosis are frequently absent in elderly persons with this disease, screening by laboratory tests is necessary for diagnosis.

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