

CASE REPORT

BARTONELLA HENSELAE: FIRST REPORTED ISOLATE IN A HUMAN IN THAILAND

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Abstract. This is the first reported case of bacillary angiomatosis associated with *Bartonella henselae* in Thailand. The clinical, pathological, and microbiological findings are presented. The bacterium was isolated from a biopsy of skin lesions obtained on admission and identified by cellular morphology, characteristics of colonies on chocolate agar, extensive biochemical tests and 16S ribosomal DNA sequencing.

INTRODUCTION

Bartonella is a genus of slow-growing, fastidious gram-negative bacteria that may necessitate a month or longer for culture isolation depending on species and individual isolates (Berbari *et al*, 1997). The genus now includes several species because all the members of the genus *Rochalimaea* were transferred into this genus (Winn *et al*, 2006). Studies conducted over the past 10 to 15 years establish that *Bartonella* constitutes a unique group of bacteria found in a wide variety of wild and domestic animals. These animals may be asymptomatic or may develop significant disease, including chronic bacteremia. Infected animals serve as reservoirs and potential sources for additional animal and human infections (Breitschweerd and Kordick, 2000). *B. henselae* (formerly *Rochalimaea henselae*) is the organism which causes bacillary angi-

omatosis (BA) in immunocompromised patients and cat-scratch disease in immunocompetent patients. BA is characterized by the eruption of cutaneous and subcutaneous nodules. As the number of lesions increases, patients may develop fever, sweats, chills, poor appetite, vomiting or weight loss. If untreated, patients may die from the complications of this disease. It is often misdiagnosed because it may be mistaken for other conditions such as Kaposi sarcoma. BA can cause blood vessels to grow out of control and form tumor-like masses in skin, bone, liver and other organs. *B. henselae* is globally endemic; serologic studies indicate that infection of domestic cats is worldwide, with the prevalence of antibodies being higher in warm, humid climates (Slater and David, 2005). *B. henselae* can multiply prodigiously in the cat flea, and the organism can remain viable in flea feces for at least 3 days (Finkelstein *et al*, 2002; Winn *et al*, 2006). Some reports from Thailand showed that *B. henselae* was isolated from domestic cats and cat fleas but no isolates were found in humans (Maruyama *et al*, 2001; Parola *et al*, 2003). We report a case of BA caused by

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B. henselae which was identified from skin lesions.

CASE REPORT

A 40-year-old woman from Myanmar was diagnosed as having symptomatic HIV infection with CMV retinitis for 3 years. Her CD4 cell count was 11 cells/mm³. She was given stavudine, lamivudine and nevirapine but her adherence to the regimen was poor. Nine months before this admission in August, 2006 she stopped her antiretroviral therapy because she went back to her country. The CD4 cell count before stopping was 178 cells/mm³. This time she came to Rajavithi Hospital because she had prolonged fever for 2 months and had lost 10 kilograms. One week prior to admission the fever increased and she noticed multiple red nodules on her face and over her entire body (Fig 1). She denied cat exposure, however, she had walked across the Thai-Myanmar border and stayed in the jungle for some nights.

Blood tests indicated a hemoglobin level of 9.7 g/dl, hematocrit of 29.1%, white blood cell count of 4,600/μl, and platelet count of 7,000/μl. The liver function test revealed an elevated alkaline phosphatase of 440 U/l (normal range; 39-117 U/l), but other tests were normal. A skin biopsy was done for pathological examination and culture. The pathology revealed nodules consisting of lobules of capillaries lined by a plump endothelium with clear cytoplasm. Mild atypia and occasional mitotic figures were present. The interstitium contained a neutrophilic infiltrate and nuclear debris. Warthin-Starry silver stain showed clumped rod-shaped organisms. BA was diagnosed and doxycycline prescribed. The lesions responded to the doxycycline. Within 2 weeks the lesions were inactive (Fig 2), and the platelet counts returned to normal at 6-weeks follow-up. Skin biopsies, bone marrow and blood cultures were collected to identify



Fig 1–The patient before treatment (with permission from the patient).



Fig 2–Two weeks after treatment.

the pathogenic bacteria using standard microbiological techniques (Thomson and Miller; 2003). For the skin biopsy and bone marrow, a conventional culture method was used. For the blood culture, the patient's blood (8-10 ml) was inoculated into the automate Bactec system (Becton Dickinson, USA), resin containing media, in order to enhance isolation of pathogenic bacteria.

Organism identification and sequencing of 16S rDNA

The skin culture yielded a pure growth of bacteria on chocolate agar after 7 days of incubation at 35°C in a 5% carbon dioxide incubator. The colonies were translucent, colorless, and circular, approximately 0.5 mm in diameter (Fig 3). The organism was a typically capnophilic bacteria (growth required a higher-than-atmospheric level of carbon dioxide), because it did not grow in an ambient incu-



Fig 3—*Bartonella henselae* tiny colonies on chocolate agar after 7 days of incubation at 35°C in the 5% carbon dioxide incubator.

bator. The organism did not grow on blood agar or MacConkey agar after 10 days of incubation at 35°C in a 5% carbon dioxide incubator. A Gram stain of the colonies from the chocolate agar revealed small gram-negative bacilli with pleomorphism (coccobacilli, plump or slightly curved rods). The results of the blood culture (13 total specimens) and bone marrow culture (1 specimen) from this patient were negative.

On biochemical tests, we found *B. henselae* has inert properties. It was negative on oxidase, catalase, urease, arginine dihydrolase, ornithine decarboxylase, and nitrate reductase tests. The various sugar fermentations in cysteine trypticase agar (CTA) *ie*, glucose, inositol, lactose, maltose, mannose, mannitol, raffinose, salicin, sorbitol, sucrose, trehalose, and xylose were negative. The substrate digestion of esculin and gelatin were also negative. The growth study on triple sugar iron agar, in 6.5% sodium chloride broth, or tryptone broth was negative. Sugar fermentation, substrate digestion and growth studies, were also tried by adding a few drops of sterile horse serum (Gibco, USA) into various media as a growth supplement for enrichment. However, the results were also negative.

Based on cellular morphology and biochemical tests described previously (Welch and Slater; 2003), our organism was identified as *Bartonella* species. In our clinical microbiology laboratory, the commercial identification system used for non-fermentative bacteria (the API 20 NE) and the automate Vitek II system (both from bioMerieux, France) were available but they do not contain *Bartonella* in their database.

We attempted to confirm the identification of this organism by molecular technique using sequencing of 16S rDNA. Extraction of the bacterial genomic DNA, amplification of the 16S rDNA by polymerase chain reaction, and subsequent sequencing of the amplicons were performed as described previously (Edwards *et al*, 1989; Eden *et al*, 1991; Weisburg *et al*, 1991; Kolbert *et al*, 2004). This strain was amplified and sequenced, and the sequence data of 978 base pairs were queried to the GenBank for best matches. The homology search result confirmed this organism as *B. henselae*, as shown in Fig 4.

DISCUSSION

BA is a systemic disease first described in a 32-year-old patient with presumed acquired immunodeficiency syndrome (AIDS) who developed multiple subcutaneous nodules characterized histologically by vascular proliferation. Bacillary forms were demonstrated, and the patient responded to erythromycin with an apparent cure (Stoler *et al*, 1983). Later, in a report that stimulated widespread attention to BA, 6 AIDS patients developed unusual cutaneous papules and nodules distinct from Kaposi sarcoma (Cockerell *et al*, 1987). The researchers, being unaware of its infectious origin, called the lesion epitheloid angiomatosis. This name was selected because the deeper dermis has alterations similar to those of epitheloid hemangiomas, with endothelial cells adherent to one

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BhsM40SHD : AGAGTTT*GATCCTGGCTCAGAACGAACGCTGGCGGCAGGCTTAAACACATGCAAGTCGAGCGCACTCTTTTAGAGTGAGCG
Th_Bhens  : -----*-----20-----*-----40-----*-----60-----*-----80-----
BhsM40SHD : GCAAACGGGTGAGTAACCGTGGGAATCTACCCATCTCTACGGAATAACACAGAGAAATTTGTGCTAATACCGTATACGT
Th_Bhens  : -----*-----100-----*-----120-----*-----140-----*-----160-----
BhsM40SHD : CCTTAGGGAGAAAGATTTATCGGAGATGGATGAGCCCGGTTGGATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGCGC
Th_Bhens  : -----*-----180-----*-----200-----*-----220-----*-----240-----
BhsM40SHD : ACGATCCATGACTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGT
Th_Bhens  : -----*-----260-----*-----280-----*-----300-----*-----320-----
BhsM40SHD : GGGGAATATTGGACAATGGGGCAACCCGTATCCAGCCATGCCCGCTGAGTGATGAAGGCCCTAGGGTTGTAAGACTCTT
Th_Bhens  : -----*-----340-----*-----360-----*-----380-----*-----400-----
BhsM40SHD : TCACCGGTGAAGATAATGACGGTAACCGGAGAAGAGCCCGGCTAACTTCGTGCCAGCAGCCCGGTAATACGAAGGGG
Th_Bhens  : -----*-----420-----*-----440-----*-----460-----*-----480-----
BhsM40SHD : GCTAGCGTTGTTCCGATTTACTGGCGTAAAGCGCATGTAGGCGGATATTTAAGTCAGAGTGAAATCCAGGGCTCAAC
Th_Bhens  : -----*-----500-----*-----520-----*-----540-----*-----560-----
BhsM40SHD : CCTGGAAGTGCCTTTGATACTGGGTATCTTGTAGTGTGGAAGAGGTGAGTGGAAATTCGAGTGTAAGGTAATAATTCGTAG
Th_Bhens  : -----*-----580-----*-----600-----*-----620-----*-----640-----
BhsM40SHD : ATATTCGGAGGAACACCAGTGGCGAAGCGGCTCACTGGTCCATTACTGACGCTGCGAAGCGTGGGGAGCAAAC
Th_Bhens  : -----*-----660-----*-----680-----*-----700-----*-----720-----
BhsM40SHD : AGGATTAGATACCCTGGTAGTCCACGCCGTAACAGTGAATGTTAGCCGTCGGGCGGTTTACTGCTCGGTGGCGCAGCTA
Th_Bhens  : -----*-----740-----*-----760-----*-----780-----*-----800-----
BhsM40SHD : ACGCGTTAAACATTCGCCCTGGGAGTACGGTGCGAAGATTTAAACTCAAAGGAATTGACGGGGCCCGCACAAGCGGTG
Th_Bhens  : -----*-----820-----*-----840-----*-----860-----*-----880-----
BhsM40SHD : GAGCATGTGGTTAATTGGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGAAGGTGGAGACACCCT
Th_Bhens  : -----*-----900-----*-----920-----*-----940-----*-----960-----
BhsM40SHD : CCTTCAGTTAGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCTGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGC
Th_Bhens  : -----*-----980-----*-----1000-----*-----1020-----*-----1040-----
BhsM40SHD : AACGAGCGCAACCCCTCGCCCTTAGTTGCCAGCATTTGGTTGGGCACTCTAGGGGGACTGCCGGTGATAAGCCGAGAGGAA
Th_Bhens  : -----*-----1060-----*-----1080-----*-----1100-----*-----1120-----
BhsM40SHD : GGTGGGGATGACGTCGAAGTCCATGGCCCTTACGGGCTGGGTACACAGTGTACAATGGTGGTGACAGTGGGCAGCG
Th_Bhens  : -----*-----1140-----*-----1160-----*-----1180-----*-----1200-----
BhsM40SHD : AGACCGGAGGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTCGGAAT
Th_Bhens  : -----*-----1220-----*-----1240-----*-----1260-----*-----1280-----
BhsM40SHD : CGTAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCGTCACACCATGGGAGTT
Th_Bhens  : -----*-----1300-----*-----1320-----*-----1340-----*-----1360-----
BhsM40SHD : GGTTTTACCCGAAGGTGCTGTGCTAACCGAAGGGGGCAGGCAACCACGGTAGGGTCAGCGACTGGGGTGAAGTCGTAAC
Th_Bhens  : -----*-----1380-----*-----1400-----*-----1420-----*-----1440-----
BhsM40SHD : AAGGTAGCCGTAGGGGAACCTGTGGCTGGATCACCTCCTT
Th_Bhens  : -----*-----1460-----*-----1480-----

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Fig 4–Alignment of 16S rRNA genes: the 16S rRNA gene segment from the Thai clinical isolate has been amplified by PCR, and the obtained DNA sequence was identical (100%) to the 16S rRNA gene sequence for *Bartonella henselae* strain M40SHD, GenBank accession number DQ645426. The universal primer annealing sites are underlined.

another in an epitheloid pattern. Its bacterial origin was finally confirmed and the name bacillary angiomatosis was proposed, originally a transitional term pending proper identification of the causative agents (LeBoit *et al*, 1989; LeBoit, 1990). In the early 1990s, several unusual clinical entities, particularly BA, were described in patients with HIV infection (Winn *et al*, 2006). BA is caused by at least 2 *Bartonella* species, *B. henselae* and *B. quintana* (formerly *Rochalimaea quintana*). The infection results from exposure to flea-infested cats for *B. henselae* and the human body louse for *B. quintana*. BA may be life threatening without therapy. It may involve either the skin, visceral organs, or both, and may be accompanied by systemic symptoms. Besides BA, *B. henselae* infection may present with prolonged fever alone, culture-negative endocarditis (De La Rosa *et al*, 2001) or cat-scratch disease for which the causative agent is not found; the patients respond to macrolide antibiotic which is widely used empirically in Thailand.

This report describes a rare case of BA caused by *B. henselae* in an HIV-infected patient. In Thailand, a case of BA and *Mycobacterium* infection coexisting in a cutaneous lesion in a patient with AIDS was reported (Chiewchanwit *et al*, 1996). However, the diagnosis was based on Warthin-Starry silver stain on sectioned biopsy and no culture was obtained. No case of BA with bacterial culture confirmation has been reported in Thailand.

The reservoir of *B. henselae* is usually the domestic cat. From a report in northern California, USA, 39.5% of 205 cats had *B. henselae* bacteremia; 52% of these bacteremic cats had more than 1,000 colony forming units/ml of blood cultured. Among these animals, 81% tested positive for *B. henselae* antibodies, with bacteremic cats having higher antibody titers than nonbacteremic cats (Chomel *et al*, 1995). In France, blood cultures 53% of 94 stray cats were positive for bacteremia with *Bartonella*

species (Heller *et al*, 1997). In the Netherlands, 50% of 113 cats were seropositive for *Bartonella* species; 22% of the cats also had positive blood cultures (Bergmans *et al*, 1997). Seven of 27 DNA samples extracted from cat fleas removed from these cats contained *Bartonella* DNA. In the USA, *B. henselae* DNA was found in 34% of 132 fleas removed from 47 cats, 89% of whom were bacteremic with *B. henselae* (Chomel *et al*, 1996). The patient in this case walked across the Thai-Myanmar border and stayed in the jungle for some nights. It is possible that transmission of *B. henselae* occurred by insect bite from insects infected with this organism in the environment.

The seroprevalence of *B. henselae* in Thai immunocompetent persons had been reported as 5.5% (Maruyama *et al*, 2000). One study showed that *B. henselae* could be isolated from domestic cats in every region in Thailand (Maruyama *et al*, 2001); hence, we believe that *B. henselae* infections in Thailand are under reported.

BA occurs most frequently in the later stages of HIV infection. In one study of 42 patients, the median CD4 cell count was 21 cells/mm³ (range: 1-228) at the time of diagnosis of BA (Mohle-Boetani *et al*, 1996). Our patient also had advanced HIV infection.

An antibiotic trial treating cats experimentally infected with *B. henselae* found a statistically significant decrease in levels of bacteremia after treatment with tetracycline or erythromycin (Regnery *et al*, 1996). The clinical response to doxycycline in this case also supported the role of *B. henselae* as the pathogen.

B. henselae is a slow growing bacteria and routine bacterial culture protocols rarely allow this organism to be detected. Making a correct laboratory diagnosis from blood culture in this case was difficult because its slow growth may not achieve established thresholds for detection in an automated blood culture system. There is a suggestion that

periodic subculture and acridine orange staining of blood culture media from an automated blood culture should be done (Spach *et al*, 1995). For other clinical specimens, such as skin biopsy or bone marrow, we usually keep the primary plate cultures (the first media plates, such as blood agar, chocolate agar and MacConkey agar in which a specimen is inoculated on the day it arrives at the clinical microbiology laboratory) in the appropriate incubators (ambient, 5% carbon dioxide or anaerobic incubator) up to 4 days before we discard the plates after observing no growth. We incubate the primary plate culture longer if a physician notifies the laboratory personnel of a suspected organism or writes the clinical diagnosis on a laboratory request form. During prolonged incubation at 35°C, the primary plate culture must be wrapped in a plastic bag or kept in a tight container to prevent drying of culture medium.

Regarding *B. henselae* identification, several methods are available, including phenotypic methods (cellular morphology on Gram stain, colonial morphology and biochemical tests), chemotaxonomic methods using fatty acid analysis in bacterial cells, and molecular techniques (*eg*, polymerase chain reaction, 16S rDNA sequencing). Definitive identification is best accomplished by molecular techniques (Winn *et al*, 2006).

In summary, we recommend that clinicians should be aware of possible infection with this organism in patients with compatible syndromes. A special culture technique should be requested, which with molecular techniques, will help identify patients with *Bartonella* infection.

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