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BARTONELLA (ROCHALIMAEA) QUINTANA CAUSING FEVER AND BACTEREMIA IN AN IMMUNOCOMPROMISED PATIENT WITH NON-HODGKIN'S LYMPHOMA

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Summary

A 48-yr-old man with stage IV non-Hodgkin's lymphoma, became neutropenic following chemotherapy and developed a fever. His blood cultures were processed to enhance the yield of fastidious bacteria. A slow-growing, capnophilic Gram-negative rod was isolated. The febrile episode was treated with cefotaxime, imipenem and vancomycin and resolved. The bacterial isolate was identified as *Bartonella (Rochalimaea) quintana* by 16S-*r*DNA gene sequencing. The isolate showed 99.8% sequence homology with the type strain.

This is the first isolation of *Bartonella (Rochalimaea) quintana* from a bacteremic patient in Australia. This bacterium is a fastidious Gram-negative rod requiring prolonged culture for its isolation. Patients with culture-negative pyrexia, especially immunocompromised patients, may need to be investigated for infection with this agent.

Key words: Bartonella (Rochalimaea) quintana, bacteremia, fastidious organism, immunocompromised.

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INTRODUCTION

Bartonella (Rochalimaea) quintana is a fastidious, slow-growing Gram-negative bacillus. This organism is recognized as the etiological agent of Trench Fever, a debilitating louse-borne illness which caused significant morbidity amongst troops during the First World War.[1] More recently *Bartonella (Rochalimaea)* species have been associated with non-epidemic clinical conditions including bacillary angiomatosis,[2-6] bacillary peliosis hepatis,[2,3] bacteremia,[2,7,8] endocarditis,[9,10] chronic lymphadenopathy[11] and cat-scratch disease.[12-16] Recent taxonomic studies have indicated that 4 species previously designated *Rochalimaea* should be reclassified into the genus *Bartonella* and removed from the order Rickettsiales.[17] These species now become *B. quintana, B. vinsonii, B. henselae* and *B. elizabethae*.

To date there have been no reported isolations of *Bartonella* sp. from blood cultures in Australia. This is probably due to the lengthy incubation periods required and to the difficulty in identifying these organisms because of their lack of biochemical activity. We report a case of bacteremia in a febrile, immunocompromised patient at the Royal Melbourne Hospital which was shown by 16S-*r*DNA gene sequence studies to be *Bartonella (Rochalimaea) quintana*.

CASE REPORT

A 48-yr-old Caucasian male presented in November 1993 with a 4 mth illness involving fever, night sweats, loss of weight (15-20 kg), watery diarrhea, intermittent joint pain and maculopapular rash progressively extending over his entire body. The patient had emigrated from Spain in 1971, returning for holidays in 1991 and 1992 with associated excursions to Italy, France and Thailand. There was no history of animal contact. His medical history included a thoracotomy in 1975 as treatment for a spontaneous pneumothorax, rheumatoid arthritis from 1979, successfully managed with intramuscular gold and methotrexate and a proximal interphalangeal joint fusion of the left fourth toe in 1992.

On examination axillary lymph nodes and liver were enlarged. The lymph node biopsy showed immunoblastic non-Hodgkin's lymphoma. Liver, bone marrow and skin were also involved (stage IV). Microbiological investigations (4 blood culture sets and 2 midstream urine cultures) were negative. HIV antibody was negative. No antibiotics were administered at this time. He underwent 3 cycles of cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) to achieve remission. During the first cycle he developed fever, sore throat, bilateral leg rashes and neutropenia. Four blood cultures and 2 midstream urine cultures were taken prior to the commencement of antibiotics. He then received 2 doses of cefotaxime 1 g initially followed by imipenem and vancomycin for 5 days.

One mth after remission he was prepared for autologous stem cell transplantation by a course of carmustine, etoposide, cytosine arabinoside and melphalan delivered via a Hickman catheter. Peripheral blood stem cells were infused with concomitant hydrocortisone. Prophylactic antibiotics, ceftazidime and vancomycin, were begun at the onset of neutropenia (day 4) post-infusion and administered for 8 days. Because he was seropositive for *Varicella Zoster* and *Herpes simplex* viruses, he also received prophylactic acyclovir. During this admission 2 blood culture sets were taken because of a febrile reaction following platelet transfusions.

In total the patient had 10 blood culture sets taken during his presenting illness and subsequent treatment. The first 4 blood culture sets obtained during the initial diagnostic work up were negative for pyogenic bacteria after a standard 5 day blood culture protocol. The 6 subsequent blood culture sets were subjected to extended incubation. Three of the 4 blood culture sets collected during the febrile neutropenic phase of his first CHOP regimen (but prior to antibiotic administration) were positive for *Bartonella* after 13 days incubation.

MICROBIOLOGY

Blood was collected from the patient during a 48 hr period and inoculated into 4 sets of Bactec NR660 blood culture media (26A and 27N, Becton Dickinson Diagnostic Instrument Systems, Maryland, USA). Bottles were incubated and processed according to a standard 5 day protocol. As the bottles failed to show a positive growth index by day 5, the aerobic vials were further subcultured onto chocolate agar as part of a project examining negative blood cultures from medical oncology patients. Agar plates were incubated at 35°C in 5% CO_2. Tiny pin-point colonies were apparent after 13 days incubation from all 3 blood specimens. At day 21 the small colonies appeared white to grey, were dry, adhered to the surface of the agar and demonstrated agar pitting. Occasional larger, smooth, tan, non-pitting colony variants were also apparent. Subcultures of these individual morphotypes onto chocolate agar led to both variants arising from the same colony. With repeated subcultures the time required for discrete colonies to appear became only 7-8 days. The organism grew only on chocolate agar at 35°C in 5% CO_2 and failed to grow on horse blood agar, sheep blood agar, human blood agar, nutrient agar and MacConkey agar in air, CO_2 or under anaerobic conditions at a range of temperatures from 4°C to 42°C. By comparison the type-strain of *B. quintana* (American Type Culture Collection VR358) grew rapidly within 5 days on chocolate agar and sheep blood agar in 5% CO_2 at 35°C. Colonial morphology of the type strain differed initially from our isolate with the type strain colonies appearing larger, smoother and more shiny and failing to pit the surface of the agar. With continued subculture our isolate of *B. quintana* developed a similar colonial morphology to that of the type strain. Gram stains of the isolate showed small, poorly staining Gram-negative bacilli. Staining with Gimenez stain showed small red bacilli, many of which were slightly curved in appearance. The organism was not acid fast.

The organism was oxidase and catalase negative and non-motile at 35°C. Inoculation of the organism into standard biochemical media[18] for urease, indole production and carbohydrate fermentation failed to yield positive results. The clinical and type strains failed to demonstrate satellitism around X factor discs (Oxoid, Basingstoke, England) on trypticase soy agar (BBL, Cockeysville, Md) in contrast to reports by previous authors.[19] A 14-day-old culture was inoculated into trypticase soy broth (BBL) containing 100 ug/mL hemin (Sigma Chemical Company, Australia) and this suspension then inoculated in duplicate into AP12ONE, API Coryne, API Strep and rapid ID32A (API Systems, France) identification strips as recommended by Drancourt and Raoult.[19] The identification strips were incubated at 35°C in air and read after 48 hrs incubation. Positive results were obtained for leucine arylamidase, arginine arylamidase, alanine arylamidase and glycine arylamidase.

As *Bartonella* sp. are relatively inert in standard biochemical tests,[20] identification of the isolate was unambiguously determined by 16S *r*DNA nucleotide sequencing. The 16S *r*DNA was amplified by the polymerase chain reaction, using primers A and H* from Edwards et al.[21] and after phosphorylation, the products were blunt-end cloned into pUC18. The complete nucleotide sequence was determined in both directions by using the internal primers described by Edwards et al.[21] and Wilson et al.[22] in conventional dideoxy-sequencing reactions.[23,24]

Assembly of the sequence data led to a contig of 1493 bp, of which the PCR primers account for 49 bp; the informative sequence was therefore 1444 bp. Comparison with reference sequences retrieved from Genbank indicated that the most significant match was with the *B. quintana* sequence (accession number M73228).[7] There was only a 3 base difference in the 1416 bp compared between our sequence and the *B. quintana* reference (=99.8% identity). Identity with the *R. henselae* (acc no M73229[7]) and *B. vinsonii* (L01259[10]) sequences was around 98.6% and with *B. elizabethae* (L01260[10]) 98.2%. Our clinical isolate was therefore identified as *B. quintana.*

DISCUSSION

Bartonella (Rochalimaea) quintana was isolated from the blood cultures of a febrile, neutropenic patient following chemotherapy for non-Hodgkin's lymphoma. This is the first recorded case of *Bartonella* species being isolated from blood cultures in Australia.

Bacteremia due to *Bartonella* sp. is probably under-reported because of the requirement for prolonged incubation periods and the failure of automated blood culture systems to detect growth of this bacterium within the usual 5 or 7 day incubation protocols recommended for these instruments. Detection of *B. quintana* using the Bactec non-radiometric system may take 4 to 6 wks after inoculation to become positive.[9] Isolation of *Bartonella* sp. from bacteremia patients may require the subcultures of blood culture broths onto solid blood enriched media such as chocolate agar or sheep blood agar. Importantly, prolonged incubation periods of 10 to 21 days are still usually required for initial growth of *Bartonella* sp. on these solid media.[4,7,10] The use of lysis-centrifugation (Isolator), the Septi-Check biphasic blood culture system and direct inoculation of blood onto solid media have all been used to isolate these organisms.[2,4,6,7,20] The use of acridine orange as a method for screening negative blood culture broths prior to discard has been shown to be effective in detecting blood cultures positive for *B. quintana.*[8]

Bartonella sp. are small, weakly staining, Gram-negative bacilli that are catalase negative. They require blood-containing media for growth and an atmosphere of 5% CO_2 for optimal growth. The oxidase test has been reported to be weakly positive using Kovacs method for *B. quintana* but negative for *B. henselae, B. vinsonii* and *B. elizabethae.*[10] The bacilli measure 1-2 $\mu m \times 0.5\ \mu m$[6] and lack flagella.[20] Microscopic detection may be more sensitive using the Gimenez stain. Using this method the bacilli stain red and have a characteristic curved morphology. Motility is variable with some strains having a "twitching" motility and others being reported as non-motile.[2,10,19]

Identification of *Bartonella* sp. can be difficult because of their inactivity in standard biochemical tests.[20] The use of a suspension of the organism in trypticase soy broth supplemented with 100 µm/mL of hemin and inoculated into the API20E, API20NE and API Coryne has led to the development of a preliminary profile for the identification of *Bartonella* species.[19] Testing for preformed enzyme activity using the commercially available kits such as the RapID

ANA II (Innovative Diagnostic Systems Inc), has been used with some success.[10,25] The Microscan Rapid Anaerobe Panel (Baxter Diagnostics) has been shown to differentiate *B. quintana* from *B. henselae* and has been suggested as an alternative method of identification to cellular fatty acid analysis, DNA methods and immunofluorescent techniques.[25] Identification of isolates has also been performed using an immunofluorescence-antibody method. Antisera to *B. quintana* and *B. henselae* have been raised in BALB/c mice by intraperitoneal inoculation and testing revealed a high specificity with no cross-reactivity between species.[6] *Bartonella* species possess a characteristic whole cell fatty acid profile which can be demonstrated using gas liquid chromatography.[20] Although this profile is similar to that of *Brucella* sp., it can be used to exclude almost all other species of bacteria.[7]

Transmission of *B. quintana* in epidemic cases of Trench Fever is considered to be via the human body louse *(Pediculus humanus)* and to involve close human contact.[1] However the mode of transmission of *Bartonella* species in other endemic cases is still unclear.[16] As in our case there are a number of reports of infection in which the patients have had no known contact with cats, body lice or other arthropod vectors. *B. quintana* has recently been isolated from the lymph nodes of a patient with adenitis and lymphopenia, suggesting a role of this organism in isolated cases of lymphadenopathy.[11] *B. henselae* which has been implicated in Cat Scratch Disease, has been isolated from the fleas of an asymptomatic bacteremic cat, confirming that arthropod vectors are carriers and that they may play a role in transmission of these organisms to humans. Further study into the epidemiology of infection with *Bartonella* species may clarify this issue.

Doxycycline, erythromycin, ceftriaxone and chloramphenicol have been used successfully in the treatment of infections with *B. quintana*.[4,27,28] The organism is exquisitely sensitive to erythromycin and ceftriaxone with reported minimal inhibitory concentrations (MIC) of $\leq$0.03 mg/L and these agents are probably the drugs of choice.[9] Our use of cefotaxime, imipenem and vancomycin has not been reported before but appears to have been successful. *In vitro* susceptibility testing of *B. quintana* to cefotaxime, and vancomycin by agar dilution has revealed MICs of <0.125 mg/L and 2 mg/L respectively.[29]

The detection of *B. quintana* bacteremia in our patient was the result of modification of existing blood culture protocols in an effort to elucidate other bacterial causes of febrile episodes in immunocompromised patients. Febrile episodes in this patient group often fail to have a microbiological cause assigned to them. It is possible in some of these cases that fastidious organisms not detected by standard blood culture protocols may be responsible. The extended incubation of blood cultures followed by routine subculture and/or acridine orange staining of "no growth" bottles, may lead to an increased detection of fastidious micro-organisms such as *Bartonella* sp. in febrile immunocompromised patients and in patients with endocarditis. Communication between the clinician and diagnostic laboratory is crucial to ensure that these techniques are appropriately utilized in these patients.

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