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Cat Scratch Disease Caused by *Bartonella grahamii* in an Immunocompromised Patient

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***Bartonella grahamii* colonizes rodents worldwide and has been detected in questing *Ixodes ricinus* ticks. Here, the first human *B. grahamii* infection confirmed by multilocus sequence typing is reported. The route of transmission and clinical picture of the patient are similar to those seen in patients with cat scratch disease, which is typically diagnosed as a *Bartonella henselae* infection.**

CASE REPORT

A 57-year-old female patient was diagnosed in February 2008 with chronic lymphocytic leukemia (CLL) with deletion of the short arm of chromosome 17 (del 17p), indicating a poor prognosis without stem cell transplantation. She was treated with fludarabine, cyclophosphamide, and rituximab from February through April 2008 with a complete response in the bone marrow but stable lymphadenopathy. Alemtuzumab was given as a second-line treatment from June through October 2008 with a variable minimal response in the lymph nodes. Allogeneic stem cell transplantation from a matched unrelated donor was performed after reduced intensive conditioning (RIC) in October 2008. RIC consisted of a combination of fludarabine, treosulfan, and antithymocyte globulin. The patient showed no symptoms of acute graft-versus-host disease (GVHD). Two months later, in December, she developed a high fever and cytomegalovirus (CMV) was detected in her blood at 2,000 copies/ml. She recovered with 4 weeks of valganciclovir treatment at 900 mg twice daily. Immunosuppressive therapy with cyclosporine was given at 125 plus 150 mg daily. She also received prophylactic valaciclovir at 500 mg twice daily, double-strength cotrimoxazole twice a day two times a week, and daily vitamin C and iron substitution therapy. Whole-body computed tomography showed axillary, cervical, and mediastinal lymph node masses, which were clearly diminished in size compared to the initial imaging before induction treatment. At the end of January 2009, her CD4 cell count was only 15×10^6/liter (4% lymphocytes), indicating a high risk of opportunistic infection. There were no signs of GVHD. During a checkup by a hematologist on 11 February 2009, the dose of cyclosporine was decreased to 150 mg daily and then to 125 mg daily after a couple of weeks. During the same visit, she again showed CMV viremia at 700 copies/ml. Valganciclovir was restarted at a dose of 900 mg twice daily. Bone marrow aspiration showed morphological remission with minimal residual disease of 0.06% by flow cytometry and 0.3% by PCR.

On 5 March 2009, the patient noticed a swollen firm lymph node in the left axilla. On admission (7 March), her temperature was 37.4°C. The patient reported a cat scratch on her left thumb a few weeks earlier. The scratch developed to a reddish papule 1 cm high and 2 cm wide at the base of her left thumb, giving an impression of an abscess with a blackish crust on it. A slight redness and inflammatory lymphadenitis in the arm and a firm painful lymph node 3 cm in diameter in the axilla were noticed. Clinically, cat scratch disease (CSD) was suspected. The abscess on her left thumb was opened and drained, and a biopsy specimen was obtained. Daily azithromycin (250 mg) was started, and the immunosuppressive treatment with cyclosporine was again slightly decreased to a daily dose of 100 mg. Computed tomography of the whole body showed axillary lymph nodes on both sides with maximal measurements of 4.2 by 2 cm on the left side and 3.6 by 2.6 by 4.9 cm on the right side. In addition, there was a hypodense, blurry-edged lesion located centrally in the right lobe of the liver. Blood tests showed a hemoglobin level of 109 g/liter, a thrombocyte count of 198×10^9/liter, and a leukocyte count of 9.2×10^9/liter with a normal differential count, liver function tests within normal limits, a C-reactive protein level of 27 mg/liter, and an erythrocyte sedimentation rate of 73 mm/h. Her serum was negative for antibodies against *Toxoplasma gondii*, *Francisella tularensis*, *Bartonella henselae*, and *Bartonella quintana* on 9 March but was not tested for these afterward. The *Bartonella* kit used was the *Bartonella* Indirect Immunofluorescence Assay IgG, IF1300G, Focus Diagnostics, Cypress, CA. The manufacturer recommends 1:64 as the starting dilution in serum screenings. Because of the heavy immunosuppressive treatment that had been given to her and also because of her CMV viremia, a 1:8 starting dilution was used. The test titer was <1:8. Blood cultures for bacteria and mycobacteria remained negative. Blood specimens for were negative CMV and Epstein-Barr virus by PCR assay. Microscopic examination of the biopsy specimen revealed hemorrhagic necrotic tissue around the ulceration of the surface of the skin consistent with

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TABLE 1 Oligonucleotide primers used for MLST

Oligonucleotide primer	Target genetic marker, oligonucleotide orientation	Sequence (5′→3′)	Reference
fD1mod	16S rRNA gene, forward	AGAGTTTGATCYTGGYTYAG	This study
fD1	16S rRNA gene, forward	AGAGTTTGATCCTGGCTCAG	1
rP2	16S rRNA gene, reverse	ACGGCTACCTTGTTACGACTT	1
Bart/16-23F	16S-23S rRNA intergenic spacer region (ISR), forward	TTGATAAGCGTGAGGTCGGAGG	5
Bart/16-23R	16S-23S rRNA intergenic spacer region (ISR), reverse	CAAAGCAGGTGCTCTCCCAG	5
BhCS.781p	GltA gene, forward	GGGGACCAGCTCATGGTGG	4
BhCS.1137n	GltA gene, reverse	AATGCAAAAAGAACAGTAAACA	4
prAPT0244	RpoB gene, forward	GATGTGCATCCTACGCATTATGG	This study
prAPT0245	RpoB gene, reverse	AATGGTGCCTCAGCACGTATAAG	This study

a histological diagnosis of bacillary angiomatosis/pyogenic granuloma. Warthin-Starry silver staining demonstrated the presence of small coccobacilli in patchy infiltrates. The fluid aspirated from the abscess was cultured for bacteria, mycobacteria, and fungi and was negative for all of them. A DNA sample was prepared from the abscess fluid with the High Pure PCR template preparation kit (11796828001; Roche). A panbacterial PCR assay with oligonucleotides fD1mod and rP2 (1) (Table 1), targeting the 16S rRNA gene, and DyNAzyme II DNA polymerase (F-501L; Thermo Scientific) that was done according to the instructions of the manufacturer and the PCR program described in Table 2 was positive. The detection limit of the 16S rRNA PCR test is 2 CFU/μl. Sequence analysis indicated *Bartonella* infection, but identification to the species level was not possible. 16S rRNA gene sequences are highly conserved within the genus *Bartonella* and therefore not useful for robust species differentiation (2).

The patient was treated with azithromycin for 5 weeks. Thereafter, an ultrasound examination of the abdomen showed no more abnormal findings in the liver or any other organs. On 22 April, the patient's condition at a checkup was good and blood tests also showed very good results—a hemoglobin level of 108 g/liter, a leukocyte count of 4.8×10^9/liter, a neutrophil count of 1.5×10^9/liter, a thrombocyte count of 158×10^9/liter, a C-reactive protein level of <1 mg/liter, and creatinine, lactate dehydrogenase, and liver function test results within normal limits. The cyclosporine dose was no more than 25 mg daily and later, by the end of April, it was discontinued. In July 2009, a slight increase in the residual disease was detected immunophenotypically (2.2%) and 2 months later, in September, a relapse was shown on the basis of a significant increase in the number and size of the lymph nodes on whole-body computed tomography. A second allogeneic transplantation was an option. Reinduction with bendamustine and alemtuzumab was done between September 2009 and January 2010. Irradiation of the axillary and cervical lymph nodes in February and May 2010 and of the mediastinal lymph nodes in September and October 2010 was done. The response was transient, and ofatumumab was started in March 2011. The patient died in April 2011 from bronchiolitis caused by respiratory syncytial virus.

TABLE 2 PCR programs used for MLST in this study

Step	Temp (°C), duration of step with oligonucleotide primers: fD1mod + rP2	Temp (°C), duration of step with oligonucleotide primers: fD1 + rP2, Bart/16-23F + Bart/16-23R, BhCS.781p + BhCS.1137n, prAPT0244 + prAPT0245
1, Initial denaturation	94, 3 min	95, 1 min
2, Denaturation	94, 30 s	95, 30 s
3, Annealing	55, 30 s	55, 40 s
4, Extension	72, 1 min (37)[a]	72, 1 min (39)[a]
5, Final extension	72, 7 min	72, 10 min
6, Hold	4, indefinitely	4, indefinitely

[a] The value in parentheses is the number of cycles of steps 2 to 4.

In December 2012, the abscess fluid DNA preparation was reanalyzed to refine the *Bartonella* diagnosis. This analysis is part of a nationwide study where clinical samples are retrospectively analyzed for the prevalence of *Bartonella* infections with PCR-based diagnostics. First, hypervariable regions V6 to V8 of the 16S rRNA gene were sequenced from a PCR-amplified fragment (with oligonucleotides fD1/rP2 and the PCR program in Tables 1 and 2, respectively). The 485-bp sequence obtained gave 100% identity scores with 16S rRNA sequences originating from three rodent-infecting species, *B. tribocorum*, *B. grahamii*, and *B. queenslandensis*. To complement the species-discriminatory power of 16S rRNA, PCR-amplified fragments of the RNA polymerase β-subunit gene (*rpoB*) (3), the citrate synthase gene (*gltA*) (4), and hypervariable region 2 of the 16S-23S rRNA intergenic spacer region (V2-ISR) (5) were sequenced (with the oligonucleotides and PCR programs in Tables 1 and 2, respectively). BLAST homology searches were performed in January 2013. The highest sequence identity score of the 406-bp *rpoB* fragment was 100% with *rpoB* fragments of several *B. grahamii* entries, including AF165993 of the V2 type strain. The next closest hit in a type strain was *rpoB* of the CIP 105476 strain of *B. tribocorum* (AM260525), which had a sequence identity score 94.3% (383/406). The highest sequence identity scores with the 338-bp *gltA* fragment were again with *B. grahamii* entries, 98.8% (334/338) with the V2 type strain *gltA* sequence (Z70016). Four *B. grahamii* entries were even closer to the patient strain *gltA* sequence, i.e., 99.1% identity (335/338) with AB426654 of a southern red-backed vole (*Myodes gapperi*) isolate from Canada, 99.4% identity (336/338) with AB426655 of a prairie vole (*Microtus ochrogaster*) isolate from the United States, 99.4% identity (336/338) with CP001562 of a wood mouse (*Apodemus sylvaticus*) isolate from Sweden, and 99.4% identity (336/338) with JQ694003 of a bank vole (*Myodes glareolus*)-infecting strain from France. The next closest hit in a type strain was *gltA*

of the CIP 105476 strain of *B. tribocorum* (AM260525), which had a sequence identity score 95.3% (322/338). The highest sequence identity score with the 286-bp V2-ISR fragment was 99.3% (284/286) with the *B. grahamii* V2 type strain ISR (AJ269785). The next closest hit in a type strain was ISR of the AUST/NH12 strain of *B. queenslandensis* (EU111765), which had a sequence identity score of 51.7% (148/286). MLST demonstrated unequivocally that the patient was infected with *B. grahamii*.

Discussion. *B. grahamii* was first isolated from the blood of a bank vole in the United Kingdom (6). Subsequent field studies have shown that this hemotrophic bacterium colonizes a multitude of rodent species worldwide in countries such as Sweden, Denmark, Poland, Spain, Slovenia, Russia, China, Thailand, Japan, Canada, and the United States (7–13). The rodent flea *Ctenophthalmus nobilis* is a robust vector for the transmission of *B. grahamii* to new rodent hosts (14). Arthropod-mediated transmission to humans also appears plausible, as *B. grahamii* was recently detected in questing *Ixodes ricinus* ticks in Germany (15). Given the environmental prevalence of *B. grahamii*, it is surprising that a confirmed human infection has remained elusive. Here, the first MLST-confirmed human *B. grahamii* infection is reported.

The first indication that *B. grahamii* may infect humans was obtained in 1999 from a case of neuroretinitis (16). 16S rRNA-specific PCR analysis of a DNA sample prepared from anterior chamber fluid yielded a sequence fragment similar to *B. grahamii*. However, this 16S rRNA fragment is 99% identical to most of the species in the genus *Bartonella* and 100% identical to rat-infecting *B. tribocorum* and *B. queenslandensis*. 16S rRNA gene sequences are highly conserved within the genus *Bartonella*, and with today's knowledge, MLST approaches are necessary to differentiate species (2). The second indication of a human *B. grahamii* infection was obtained from a case of bilateral retinal artery branch occlusions in an immunocompetent patient (17). His serum was IgM positive (titer of 1:50) for *B. grahamii* but negative for *B. henselae* and *B. quintana*, indicating that the patient had recently been exposed to a *B. grahamii*-like bacterium. The first MLST-confirmed patient case, reported here, demonstrates that *B. grahamii* may indeed cause human infections.

The clinical picture of the patient was similar to that of a patient with CSD, which is typically diagnosed as a *B. henselae* infection (18, 19). However, enlarged lymph nodes were revealed by computed tomography of the patient also without the *Bartonella* infection that is typical of CLL. Serial imaging helped to detect the infection and differentiate the situation from a CLL relapse. Imaging also showed a hypodense lesion in the liver of the patient and that the lesion disappeared after antibiotic therapy. The patient was severely immunosuppressed, which may have influenced the clinical picture and allowed a different *Bartonella* species to cause CSD symptoms. Given its environmental prevalence, *B. grahamii* could also cause other febrile diseases in humans. It is hard to believe that such infections are rare. It is more likely that infections do take place but remain largely asymptomatic unless the individual is immunosuppressed. Current clinical diagnostic procedures may also fail to detect the bacterium. Indeed, our patient was culture negative and seronegative. Culturing of *Bartonella* spp. is demanding, and it may take several weeks until visible colonies appear. Serology, in turn, is known to be uncertain to detect infection in severely immunocompromised patients. Clinicians should bear in mind that robust PCR-based MLST techniques for *Bartonella* spp. that allow determination to the species level are available (Tables 1 and 2) (2).

The transmission of rodent-infecting *B. grahamii* to our patient was traced to a cat scratch. It is thought that the cat had caught an infected rodent while roaming outdoors and carried *B. grahamii*-infected tissue or blood on its claws. *B. grahamii* is one of the most prevalent hemotrophic bacteria in wild rodents. Clinical and public awareness of this zoonotic threat should be increased.

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this study have been deposited in GenBank under accession no. KC633097 to KC633100.

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