Bacteremia, Fever, and Splenomegaly Caused by a Newly Recognized Bartonella Species


SUMMARY

Bartonella species cause serious human infections globally, including bacillary angiomatosis, Oroya fever, trench fever, and endocarditis. We describe a patient who had fever and splenomegaly after traveling to Peru and also had bacteremia from an organism that resembled Bartonella bacilliformis, the causative agent of Oroya fever, which is endemic to Peru. However, genetic analyses revealed that this fastidious bacterium represented a previously uncultured and unnamed bartonella species, closely related to B. clarridgeiae and more distantly related to B. bacilliformis. We characterized this isolate, including its ability to cause fever and sustained bacteremia in a rhesus macaque. The route of infection and burden of human disease associated with this newly described pathogen are currently unknown.

HUMAN INFECTION WITH BARTONELLA PROBABLY HAS OCCURRED FOR centuries, but only in the past several decades have the prevalence of infection in humans and the diversity of infecting species been recognized. In 1990, a new species called Bartonella henselae was shown to cause bacteremia and bacillary angiomatosis in patients with the acquired immunodeficiency syndrome (AIDS).1,2 Previously, there had been only two known bartonella species that infected humans: B. quintana, identified in Europe during World War I as the agent causing relapsing bacteremia in tens of thousands of troops afflicted with trench fever, and B. bacilliformis, endemic only in the Andes, where it causes a hemolytic bacteremia called Oroya fever and the angioproliferative cutaneous manifestations of verruga peruana. After the discovery of B. henselae, B. quintana was isolated from bacillary angiomatosis lesions from homeless patients with AIDS who had body lice,3,4 B. henselae was identified as the agent of cat scratch disease,5 and both species were identified as a substantial cause of culture-negative endocarditis.6

CASE REPORT

A 43-year-old American woman had a fever after traveling in Peru for 3 weeks. She visited Lima for several days, and then traveled to Nazca, where she resided in a lodge in a desert area at sea level. She then traveled to the Sacred Valley of Urubamba, followed by Cuzco and Machu Picchu, where she hiked and spent one night. Her trip concluded in the Amazon Basin near Iquitos. She received numerous insect bites, predominantly on the legs and feet.

Sixteen days after returning to the United States, the patient had fever, insomnia, myalgia, nausea, headache, and mild cough. During the first 4 days of fever, her temperature was as high as 38.9°C; it decreased during the next 3 days, but the
day before presentation she had a recurrent fever, with a maximum temperature of 38.9°C. Six days before presentation she had a diffuse macular rash. She came to the clinic 8 days after the onset of fever. Physical examination revealed a temperature of 37.3°C, an enlarged spleen palpated 4 to 5 cm below the left costal margin, and healing insect bites on the legs and feet. Microscopical examination of a peripheral-blood specimen did not show bacteria or malaria parasites within erythrocytes. Laboratory values revealed a mild anemia that resolved 6 weeks after treatment (Table 1). A blood specimen from the patient was cultured, and she was given oral levofloxacin for 5 days as empirical treatment for enteric fever. One week later, she was asymptomatic and afebrile, and the spleen was not palpable. The patient did not own pets and had not been exposed to cats during or after her travel to Peru. There was no other recent travel, and her traveling companion remained well.

**Methods**

A blood specimen from the patient was cultured in a 40-ml culture bottle (BACTEC Standard/10 Aerobic/F, Becton Dickinson) at the time of presentation. On day 15 of incubation, the automated culture system signaled a positive culture. Samples were submitted to one laboratory for Gram’s staining, acridine orange staining, and subculturing and to another for subculturing. A 5-ml aliquot of blood was centrifuged at 5000×g for 45 minutes. The supernatant and pellet were plated onto heart infusion agar containing 5% fresh defibrinated rabbit blood and chocolate agar, and the plates were incubated at 28°C or 35°C in a candle-extinction jar. Fluorescence microscopy of the positive blood-culture broth stained with acridine orange was performed with the use of an epifluorescence microscope (excitation wavelength, 450 to 490 nm; mean emission wavelength, >515 nm). Subsequently, the morphologic characteristics of the isolate were examined by means of transmission electron microscopy after negative staining.

On the basis of the presumptive identification of the isolate (designated BMGH) as a bartonella species, serum samples collected at presentation and follow-up were also assayed, with the use of class-specific conjugates labeled with fluorescein isothiocyanate, to determine reactivity against the isolate from the patient. In the IgM assays, sam-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Presentation (9/4/03)</th>
<th>Follow-up</th>
<th>Normal Range</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 Day (9/5/03)</td>
<td>6 Weeks (10/28/03)</td>
<td>18 Months (4/7/05)</td>
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<tr>
<td>Hematocrit (%)</td>
<td>32.1</td>
<td>35.6</td>
<td>37.5</td>
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<tr>
<td>Erythrocyte count (x10⁹/mm³)</td>
<td>3.8</td>
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<td>Platelet count (per mm³)</td>
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<td>474,000</td>
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<td>White-cell count (per mm³)</td>
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<td>6,700</td>
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<tr>
<td>Neutrophils (%)</td>
<td>72</td>
<td>65</td>
<td>71</td>
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<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>10</td>
<td>12</td>
<td>10–20</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8</td>
<td>0.8</td>
<td>&lt;1.5</td>
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<tr>
<td>Aspartate aminotransferase (U/liter)</td>
<td>31</td>
<td>16</td>
<td>0–35</td>
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<tr>
<td>Alanine aminotransferase (U/liter)</td>
<td>28</td>
<td>25</td>
<td>0–35</td>
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<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.0</td>
<td>1.9</td>
<td>0.3–1.0</td>
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<tr>
<td>Alkaline phosphatase (U/liter)</td>
<td>69</td>
<td>51</td>
<td>30–120</td>
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<td>Reciprocal titers of antibody against BMGH isolate by indirect fluorescence-antibody testing</td>
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<td></td>
<td></td>
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<tr>
<td>IgM</td>
<td>4,096</td>
<td>512</td>
<td>&lt;64</td>
</tr>
<tr>
<td>IgG</td>
<td>512</td>
<td>4,096</td>
<td>&lt;64</td>
</tr>
</tbody>
</table>

*To convert the values for urea nitrogen to millimoles per liter, multiply by 0.357. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for total bilirubin to micromoles per liter, multiply by 17.1.
ples were first depleted of IgG with the use of a protein-G removal device (Mini Rapi-Sep-M, Pan-Bio). A reciprocal titer of 64 or more represents a positive result for *B. henselae* or *B. quintana.*

**AMPLIFICATION BY POLYMERASE CHAIN REACTION AND SEQUENCE ANALYSIS**

DNA from the BMGH isolate was extracted, and sequencing was performed as previously described. Oligonucleotide primers (from the Centers for Disease Control and Prevention Core Facility), gene targets, and GenBank accession numbers for nucleotide sequences generated in this study are listed in Table 1 of the Supplementary Appendix (available with the full text of this article at www.nejm.org). We aligned the sequences using ClustalW software and drew the phylogenetic tree using Molecular Evolutionary Genetics Analysis software, version 3.0. The distance matrix was calculated with the use of Kimura-2 parameters, and the tree was obtained with the use of the unweighted pair group method with arithmetic mean, based on a comparison of 1328 sites.

**ONE-DIMENSIONAL AND TWO-DIMENSIONAL GEL ELECTROPHORESIS**

For one-dimensional sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS-PAGE), bacterial proteins were electrophoresed on an 8 to 16% gradient gel and stained with Coomassie blue. For two-dimensional SDS-PAGE, subcellular fractions were prepared and a protease-inhibitor cocktail was added. Cytosolic proteins were precipitated and dialyzed overnight with the use of phosphate-buffered saline and then concentrated with a centrifuge filter (Amicon Ultra-4; Millipore). Two-dimensional gel electrophoresis was then performed (by Kendrick Laboratories): isoelectric focusing was carried out in glass tubes with the use of 2% pH 4–8 ampholines (BDH) for 9600 volt-hours. The tube gels were separated on 10% acrylamide slab gels and stained with silver nitrate.

**INOCULATION OF A RHESUS MACAQUE WITH THE BMGH ISOLATE**

To evaluate the ability of the isolate to cause disease, we next inoculated a rhesus macaque previously infected with the simian immunodeficiency virus. BMGH (culture passage 2) was grown for 6 days to confluency on 5% fresh defibrinated rabbit blood agar. The plates were scraped into M199 medium with Earle’s salts and mixed, and 100 to 150 μl of the mixture (1.89×10⁸ colony-forming units per milliliter) was inoculated intradermally in each of eight separate sites. In addition, 0.8 ml of inoculum was further diluted to a final volume of 4 ml with M199 medium with Earle’s salts and was inoculated intravenously. Blood specimens from the inoculated macaque were cultured in a 2-ml EDTA tube (Becton Dickinson) twice a week and incubated at 35°C as previously described. All protocols and procedures were reviewed and approved by the institutional animal care and use committee.

**RESULTS**

A bartonella-like bacterium was isolated from a blood specimen from the patient. Fifteen days after inoculation, a positive signal was detected in the BACTEC bottle inoculated with the blood specimen. No organisms were detected on Gram’s staining, but clusters of organisms were found on acridine-orange staining (Fig. 1A). Agar plates inoculated with broth from the blood culture with a positive signal grew confluent colonies after 10 days of incubation. Small, gram-negative bacilli with multiple, unipolar flagella were visualized on transmission electron microscopy (Fig. 1B). The appearance of the BMGH isolate was indistinguishable from that of *B. bacilliformis*. Repeat blood cultures were performed 6 weeks after the first set, and the patient was treated with a second course of antibiotics, oral clarithromycin for 10 days. The follow-up cultures were negative.

Using the homologous BMGH antigen, we found a significant increase in the IgG antibody level and a significant decrease in the IgM antibody level in the serum samples at 6 weeks, each differing by a factor greater than four as compared with the serum samples at presentation (Table 1). The titers were consistent with an acute infection caused by this bartonella organism.

Comparison of the gene sequences of the 16S ribosomal DNA (rDNA), the *gltA* and *rpoB* fragments, and the 16S–23S intergenic spacer region showed that the BMGH isolate is a new bartonella species, most closely related phylogenetically to *B. clarridgeiae*. The sequence of an 1171-bp fragment of the BMGH 16S rDNA had 99.2 to 99.7% similarity to that of *B. clarridgeiae*, with nine variable nucleotide positions (four that were consistently different from those found in the five
B. clarridgeiae isolates tested) (Table 2 of the Supplementary Appendix).

Among the bartonella species, sequences of BMGH gltA and rpoB fragments were again most similar to B. clarridgeiae (95.9% and 91.7% similarity, respectively) (Table 3 of the Supplementary Appendix), a finding that is consistent with the proposed criteria for a new bartonella species.21 Searching with the use of the Basic Local Alignment Search Tool revealed that the 1439-bp fragment of the 16S–23S intergenic spacer region was most similar to that of the uncultured bartonella F17688 (GenBank accession no. AF415211), amplified from a flea (pulex species) removed from a human in Cuzco, Peru.22 The 970-bp sequence available for bartonella F17688 had 99.8% similarity with the corresponding sequence of the 16S–23S intergenic spacer region of the BMGH isolate (with only two differences detected). Phylogenetic analysis of this fragment resulted in the placement of the BMGH isolate and the uncultured bartonella F17688 in the same clade as B. clarridgeiae (Fig. 2), but the eight B. clarridgeiae isolates from different geographic regions formed a phylogenetic lineage that differed from that of the BMGH and F17688 isolates (supported by 100% of the bootstrap replications). These genetic data indicate that BMGH is a new bartonella species, which we have named B. rochalimae, that is distinct from B. clarridgeiae.

The one-dimensional protein profiles show distinct differences between BMGH and the two B. clarridgeiae isolates, two B. bacilliformis isolates, B. henselae, and B. quintana (Fig. 1G of the Supplementary Appendix). The two-dimensional protein profiles of both the cytosolic and total-outer-membrane protein subcellular fractions of BMGH (Fig. 1C and 1D in the Supplementary Appendix) also showed a profile distinct from those of B. bacilliformis (Fig. 1A and 1B in the Supplementary Appendix) and B. clarridgeiae (Fig. 1E and 1F in the Supplementary Appendix).

After experimental inoculation of the macaque with BMGH, bacteremia was detected first on day 14 and also on days 17, 22, and 24 (0.75, 1.50, 12.75, and 11.25 colony-forming units per milliliter, respectively). Isolates were confirmed to be BMGH by means of amplification and sequencing of the 16S rDNA. The animal had an increase in temperature (from 37.6°C to 38.7°C on day 3) and a decrease in hematocrit (from 40.1% to 33.9% on day 7, with a gradual increase to 38.3% by day 70).

**DISCUSSION**

The bartonella genus consisted of a single species in 1992, but there are now 19 officially recognized and extant species and subspecies. At present, humans are the sole reservoir host for only two spe-
cies of bartonella: *B. quintana* and *B. bacilliformis*. One additional species, *B. henselae*, has been isolated occasionally from immunocompromised humans, but the domestic cat is the primary mammalian reservoir for this species. Distinct epidemiologic risk factors are significantly associated with the acquisition of these three bartonella species that infect humans: homelessness and exposure to human body lice for *B. quintana*, scratches from fleas for *B. henselae*, and visits to or residence in a geographically restricted region of the Andes where the sand-fly vector is found for *B. bacilliformis*.²⁴

Our patient presented with fever, splenomegaly, anemia, and a history of insect bites after a trip to Peru that included travel to a region in which *B. bacilliformis* is endemic. On the basis of clinical, epidemiologic, and microbiologic evi-

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**Figure 2.** Phylogenetic Tree of the 16S–23S Intergenic Spacer Region in Bartonella Species.

The new isolate, BMGH, is most closely related to, but distinct from, *Bartonella clarridgeiae*. The numbers at the nodes are the percentages of 1000 bootstrap replications that support the configuration shown. Only bootstrap percentages greater than 80 are shown. The names of *B. clarridgeiae* isolates and GenBank accession numbers are shown after the species name. The homologous sequence of *Brucella melitensis* was used as the outgroup. Bartonella species isolated and propagated from humans are indicated with an asterisk.
dence, we initially assumed that the small, motile, fastidious bacterium isolated from her blood was *B. bacilliformis*. However, molecular characterization of the organism revealed a new species of *Bartonella*, closely related to, but distinct from, *B. claridgeiae*. The mammalian reservoir for *B. claridgeiae* is the domestic cat, and the prevalence of *B. claridgeiae* bacteremia in cats varies worldwide, from less than 10% in the United States to 36% in Europe.\(^{25,26}\) Despite the substantial prevalence of *B. claridgeiae* in cats, it has never been isolated in a human or amplified from human tissue specimens, including those from patients with cat scratch disease or bacillary angiomatosis.\(^4,27\)

*B. bacilliformis* has caused multiple outbreaks of Oroya fever in persons living in certain regions of Peru, Ecuador, and Colombia. Many of these persons received a diagnosis of infection with *B. bacilliformis* on the basis of clinical findings and examination of blood smears or blood cultures. Although we did not observe intraerythrocytic organisms on the blood smear from our patient, the fever, anemia, splenomegaly, and isolation of a *Bartonella* species raise the possibility that some cases of Oroya fever could represent infection with this new *Bartonella* species. It will be of interest to determine the prevalence of this new species in humans residing in the Andes and to identify whether it also can cause a form of infection similar to verruga peruana, the chronic form of infection with *B. bacilliformis*.

*B. bacilliformis* and *B. quintana* are transmitted to humans by arthropods. Our patient noted insect bites on her legs and feet during her trip to Peru, but the source insect could not be identified. Using a polymerase-chain-reaction assay, Parola et al.\(^{22}\) identified a sequence for the 16S–23S intergenic spacer region in a *Pulex* flea found on a person in Cuzco, Peru, that was nearly identical to the BMGH sequence — suggesting a possible vector.

In conclusion, we have identified a new *Bartonella* species that caused an illness with features resembling Oroya fever in a patient who had recently traveled to Peru. Whether a zoonotic reservoir exists and the mechanism by which human infection occurs are currently unknown. This case illustrates the importance of culturing specimens from patients to test for *Bartonella* species, performing careful molecular characterization of *Bartonella* isolates, and remaining vigilant for new *Bartonella* species that are pathogenic in humans.

To fulfill the rules of the International Code of Nomenclature of Bacteria (Lapage SP, Sneath PHA, Lessef EF, Skerman VBD, Seeliger HPR, Clark WA. International code of nomenclature of bacteria (1990 revision). Washington, DC: American Society for Microbiology, 1992.), we provide the following description of the novel species identified in this report. Description of *Bartonella rochalimae* sp. nov. *Bartonella rochalimae* (rocha-li-mae. N.L. gen. masc. n. *rochalimae*, of Rochalima, named in honor of Henrique da Rocha-Lima, an early Brazilian investigator of the etiology of rickettsial diseases). Small, motile, fastidious gram-negative rod with multiple, unipolar flagella that grows on fresh defibrinated rabbit-blood agar at 35°C in a candle extinction jar. *Bartonella rochalimae* is differentiated genetically from other *Bartonella* species on the basis of unique sequences of 16S rDNA, *gltA* and *rpoB* genes, and 16S–23S intergenic spacer region. The bacterium infects and is pathogenic for humans and macaques. The type strain is BMGH (available as American Type Culture Collection no. BAA-1498), isolated from a 43-year-old woman with splenomegaly, fever, anemia, and recent travel to Peru.

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Careful microbiologic evaluation of patients with various illnesses has led to the discovery of many important pathogens in recent decades, including human immunodeficiency virus (HIV), legionella species, *Borrelia burgdorferi* (the agent of Lyme disease), human herpesvirus 8 (HHV-8), and numerous others. Success in these endeavors, however, was critically dependent on the availability of the appropriate technology for both the detection of the microorganism and its characterization to the level necessary to permit clear differentiation from already recognized pathogens. The delay between the recognition of a particular clinical syndrome and the identification of its causative agent has been highly variable. Whereas HIV, for example, was discovered within 2 to 3 years after the recognition of AIDS, it took more than 120 years to establish that HHV-8 was the cause of Kaposi’s sarcoma.

Bartonella are small, curved, pleomorphic, gram-negative rods. A characteristic feature of these bacteria is their adherence to and invasion of erythrocytes, although this phenomenon is dependent on the erythrocytes’ species of origin. A unique facet of infection with Bartonella is the ability of these microorganisms to stimulate neovascular proliferation in tissues, presumably by causing endothelial-cell proliferation and migration. Although highly fastidious, Bartonella are often cultivable, and available methods for analyzing the genetic and protein compositions of the isolated microorganism permit very precise molecular characterization. Having used such an approach, Eremeeva et al. presented compelling evidence in this issue of the *Journal* (pages 2381–2387) that a new Bartonella species, *Bartonella rochalimae* sp. nov., should be added to the list of recognized human pathogens.

Of the 19 recognized and extant species and subspecies in the expanding Bartonella genus before the report by Eremeeva et al., perhaps 9 had been linked to human infections, but only 3 of them had been implicated in such infections frequently. The spectrum of clinical illness varies with the species causing the infection, but even among patients infected with the same species, the clinical features can be surprisingly variable. At times, the clinical illness caused by these microorganisms is so distinctive that Bartonella infection would be at or near the top of the differential diagnosis, whereas in other patients the presentation is completely nonspecific.

*B. henselae* is now regarded as the principal cause of cat scratch disease, the most frequently recognized Bartonella infection in humans. The cause of cat scratch disease was not conclusively elucidated until more than 40 years after its recognition as a clinical entity in 1950. The hallmark of this infection is the prominent enlargement of lymph nodes that drain lymph from cutaneous sites where *B. henselae* was introduced by the cat’s scratch. Another serious clinical consequence of *B. henselae* infection is bacillary angiomatosis, an unusual, rapidly expanding vascular neoplasm that, in the right circumstances, can cause serious morbidity (see accompanying illustration). In other cases, Bartonella infection may be associated with less overt clinical illness, such as infection of the central nervous system, where it can cause a rapidly progressive neurologic illness (see page 2875).

The list of recognized Bartonella species is expected to grow as our understanding of the biology and clinical impact of these microorganisms expands. As a guide to the identification of Bartonella species and subspecies, Eremeeva et al. provide a detailed account of the genetic and biochemical characteristics that distinguish the species from one another. Their work is an important contribution to the expanding literature on this fascinating group of microorganisms.
B. bacilliformis infection, to bleed. The infected patient described by Eremeeva et al. had a mild illness with fever and anemia; given her travel history, she would have been given a diagnosis of Oroya fever had these investigators been less conscientious about precisely identifying the bacterium recovered from her blood. Since the only closely related microorganism that had previously been recognized was found in a flea, it is conceivable that the newly recognized infection was transmitted by a vector other than a sand fly. A flea vector could mean that this infection will be distributed somewhat differently from Oroya fever. Meticulous bedside-to-bench research like that conducted by Eremeeva et al. is vital to the discovery of new or previously unrecognized infectious diseases.

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