

## BRIEF REPORT

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

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## 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.

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**H**UMAN 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).<sup>1,2</sup> 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,<sup>3,4</sup> *B. henselae* was identified as the agent of cat scratch disease,<sup>5</sup> and both species were identified as a substantial cause of culture-negative endocarditis.<sup>6</sup>

## 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<sup>2</sup> and chocolate agar,<sup>7</sup> 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.<sup>8</sup>

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 1. Laboratory Data at Presentation and at Follow-up.\***

Variable	Presentation (9/4/03)	Follow-up			Normal Range
		1 Day (9/5/03)	6 Weeks (10/28/03)	18 Months (4/7/05)	
Hematocrit (%)	32.1	33.2	35.6	37.5	36.0–46.0
Erythrocyte count ( $\times 10^{-6}/\text{mm}^3$ )	3.8	4.0	4.2	4.3	4.0–5.2
Platelet count (per $\text{mm}^3$ )	351,000	416,000	474,000	363,000	150,000–350,000
White-cell count (per $\text{mm}^3$ )	6,100	5,700	6,700	8,200	4,500–11,000
Neutrophils (%)	72	64	65	71	40–70
Urea nitrogen (mg/dl)	10			12	10–20
Creatinine (mg/dl)	0.8			0.8	<1.5
Aspartate aminotransferase (U/liter)	31			16	0–35
Alanine aminotransferase (U/liter)	28			25	0–35
Total bilirubin (mg/dl)	1.0			1.9	0.3–1.0
Alkaline phosphatase (U/liter)	69			51	30–120
Reciprocal titers of antibody against BMGH isolate by indirect fluorescence-antibody testing					
IgM	4,096		512		<64
IgG	512		4,096		<64

\* 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*.<sup>9,10</sup>

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

DNA from the BMGH isolate was extracted, and sequencing was performed as previously described.<sup>11-15</sup> 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](http://www.nejm.org)). We aligned the sequences using ClustalW software and drew the phylogenetic tree using Molecular Evolutionary Genetics Analysis software, version 3.0.<sup>16</sup> 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<sup>17,18</sup> 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<sup>19</sup> (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.<sup>20</sup>

#### 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

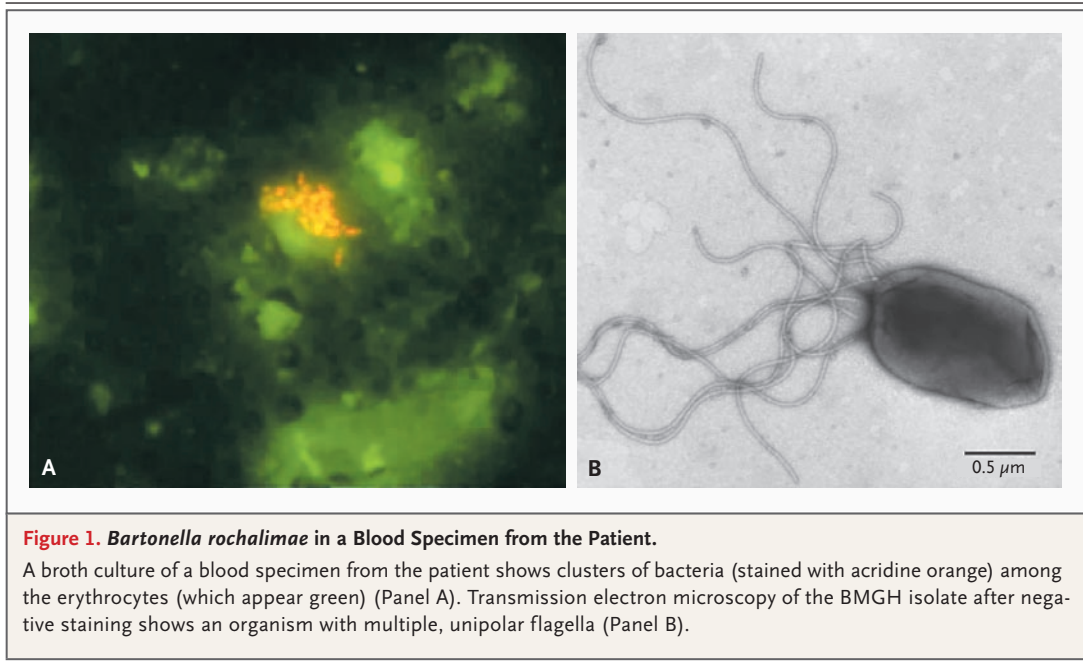
M199 medium with Earle's salts and mixed, and 100 to 150  $\mu$ l of the mixture ( $1.89 \times 10^8$  colony-forming units per milliliter) was inoculated intradermally in each of eight separate sites. In addition, 0.8 ml of inoculum was further diluted to a final volume of 4 ml with M199 medium with 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.<sup>3</sup> 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



**Figure 1. *Bartonella rochalimae* in a Blood Specimen from the Patient.**

A broth culture of a blood specimen from the patient shows clusters of bacteria (stained with acridine orange) among the erythrocytes (which appear green) (Panel A). Transmission electron microscopy of the BMGH isolate after negative staining shows an organism with multiple, unipolar flagella (Panel B).

*B. clarridgeiae* isolates tested) (Table 2 of the Supplementary Appendix).

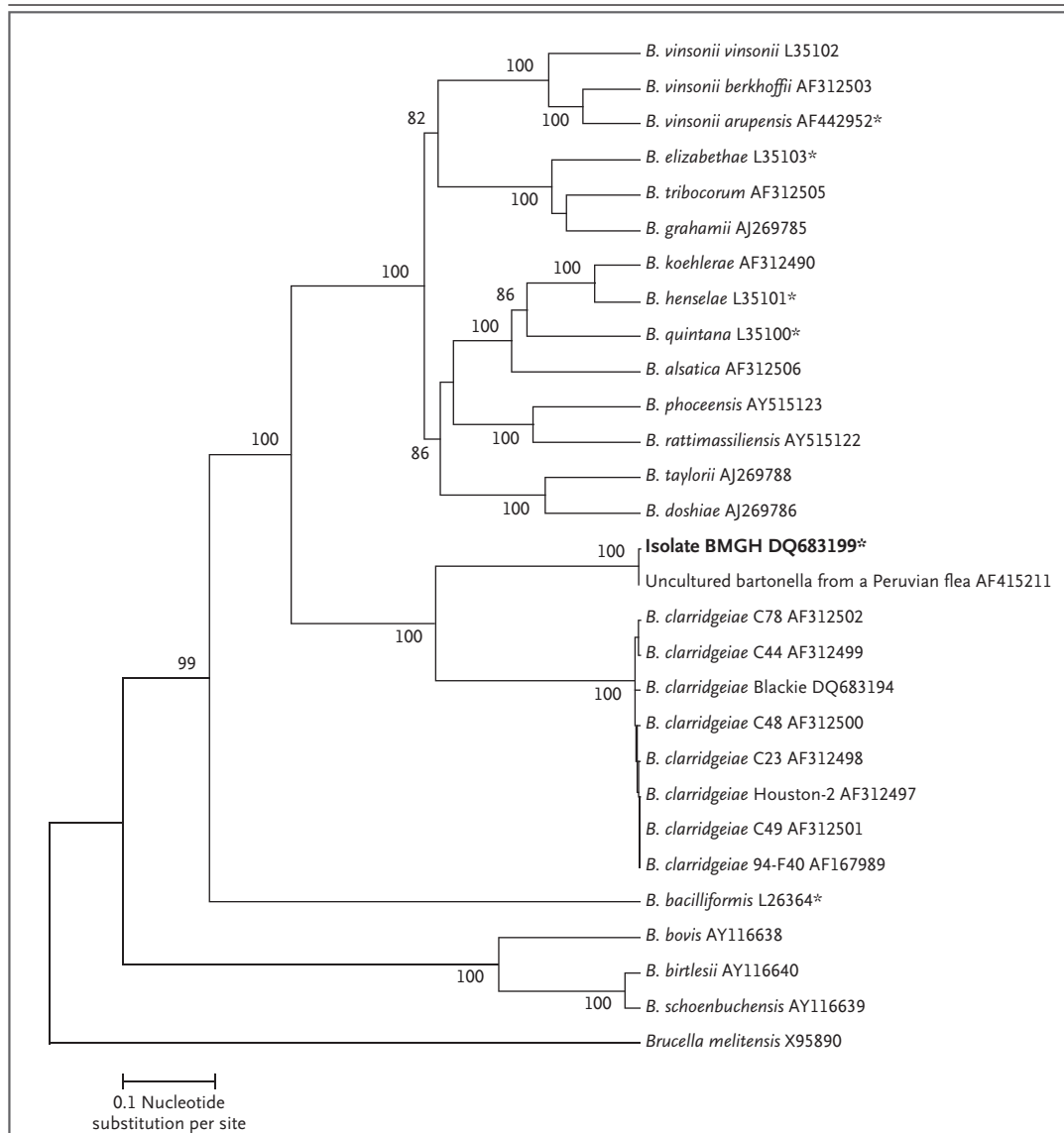
Among the bartonella species, sequences of BMGH *gltA* and *rpoB* fragments were again most similar to *B. clarridgeiae* (95.9% and 91.7% similarity, respectively) (Table 3 of the Supplementary Appendix), a finding that is consistent with the proposed criteria for a new bartonella species.<sup>21</sup> 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.<sup>22</sup> 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-



**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.

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*,<sup>4</sup> scratches from kit-

tens infested with fleas for *B. henselae*,<sup>23</sup> and visits to or residence in a geographically restricted region of the Andes where the sand-fly vector is found for *B. bacilliformis*.<sup>24</sup>

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-

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. clarridgeiae*. The mammalian reservoir for *B. clarridgeiae* is the domestic cat, and the prevalence of *B. clarridgeiae* bacteremia in cats varies worldwide, from less than 10% in the United States to 36% in Europe.<sup>25,26</sup> Despite the substantial prevalence of *B. clarridgeiae* 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.<sup>4,27</sup>

*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.<sup>22</sup> 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, Lessel 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* (ro-cha-li-ma'e. 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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No potential conflict of interest relevant to this article was reported.

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