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Meningitis Due to a "*Bartonella washoensis*"-Like Human Pathogen▽

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We report the second human case of infection caused by an organism identified as the proposed *Bartonella* species, "*B. washoensis*." The organism was isolated from a blood sample from a patient presenting with meningitis and early sepsis. *Oropsylla montana* fleas were implicated as the vector for disease transmission in this case.

CASE REPORT

A 47-year-old, previously healthy woman presented to a northern California emergency department in August 2008 with subjective complaint of 1 day of fever, chills, headache, nausea, one episode of vomiting, and epigastric and lower left side abdominal pain. In the few hours immediately preceding presentation, her headache worsened, with severe bilateral pain in the frontal and occipital areas. She also developed photophobia and bilateral joint pain in both upper and lower extremities. Upon examination, she was febrile (39.3°C) and was noted to have neck stiffness with mild nuchal rigidity. All other systems were within normal limits. She did not have confusion, blurry vision, cough, chest pain, shortness of breath, diarrhea, or rash. She had one episode of hypotension (96/50; heart rate of 104 beats per minute) in the emergency department which resolved with intravenous hydration. The patient was admitted for workup of meningitis and early sepsis. In light of a history of penicillin allergy, the patient was started on empirical vancomycin and chloramphenicol. The chloramphenicol was later discontinued, and aztreonam was started. The patient was employed as a middle school teacher and recalled no recent contacts with sick individuals. The patient had traveled to the Oregon coast for an indoor family event 1 week prior to onset. She owned 5 dogs, 1 cat, 1 calf, 2 horses, 15 sheep, and several chickens. The patient reported occasionally observing mice and ground squirrels on her property and had recently picked up a dead mole and ground squirrel with her bare hands. She denied any recent flea, tick, or other insect bites. Imaging studies, including cranial computed tomography, abdominal computed tomography, and thoracic X-ray, were normal. A complete blood count showed a hematocrit of 36.5%, platelet count of 168 × 10^3 cells/μl, and leukocyte count of 7.3 × 10^3 cells/μl with a differential of 87% neutrophils and 8% lymphocytes. Coagulation times, serum chemistries, and liver function tests were normal. Cerebrospinal fluid (CSF) obtained by lumbar puncture showed a glucose level of 64 mg/dl, total protein of 40 mg/dl, 1 red blood cell/mm^3, and 6 white blood cells/mm^3 with a differential of 51% neutrophils and 49% monocytes. Bacterial culture of the CSF sample was attempted using chocolate agar, blood agar, MacConkey agar, and thioglycolate broth. All CSF cultures were negative for growth after 5 days of incubation. PCR performed on the CSF specimen for enterovirus detection was also negative. The patient improved clinically and was discharged after 3 days on oral moxifloxacin. At discharge, a complete blood cell count showed a hematocrit of 35.3%, a platelet count of 112 × 10^3 cells/μl, and a leukocyte count of 3.5 × 10^3 cells/μl. At a follow-up visit on day 6, urinalysis showed trace leukocyte esterase, positive nitrates, zero to three red blood cells, and six to nine leukocytes. The patient was switched to oral levofloxacin for 10 days to treat a presumed complicated urinary tract infection. Eight weeks after hospitalization, the patient reported feeling clinically improved but still "not normal," with persistent body aches and bone pain.

Blood collected at admission was cultured using the BACTEC blood culture system (BD Diagnostic Systems, Sparks, MD), and bacterial growth was detected in the aerobic bottle after 5 days of incubation. Bacterial isolation was performed by subculture of the blood culture medium to chocolate and blood agar plates. The bacterial isolate, strain 08S-0475, was submitted as a possible *Capnocytophaga* sp. to the Microbial Diseases Laboratory, California Department of Public Health, for identification (the isolate has been deposited as accession number ATCC BAA-1777 with the American Type Culture Collection, Manassas, VA). Staining revealed gram-negative, medium-length rods with a thin, wavy appearance. Pinpoint colonies were observed on chocolate agar after 4 days of incubation at 35°C in 5% CO_2. The colonies were small, grayish-white, translucent, and smooth in appearance and soft when touched with an inoculation loop. The isolate grew more slowly on fresh blood agar plates containing 5% defibrinated rabbit blood incubated at 35°C in 5% CO_2. Hemolysis was not observed. No bacterial growth was observed under anaerobic conditions or when inoculated on brain heart infusion agar supplemented with calf serum. The isolate was negative for oxidase and catalase activity. Peptidase activity was assessed using the

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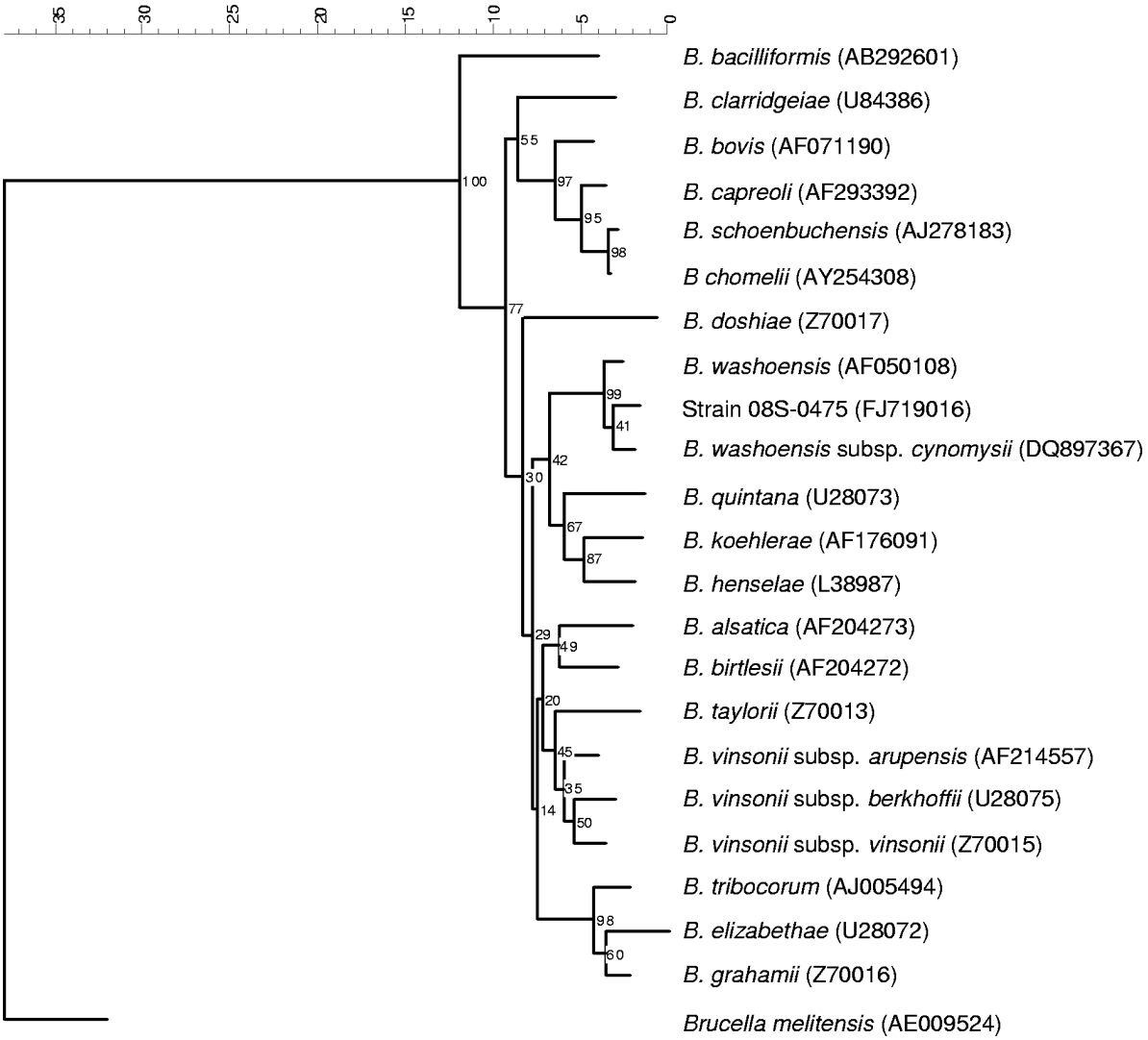

FIG. 1. Comparison of a 290-bp region of *gltA* sequences from various *Bartonella* species. The dendrogram was prepared using the neighbor-joining algorithm and Kimura's two-parameter model. Bootstrap values at branch nodes are based on 100 replicates. The cluster consisting of *B. washoensis*, *B. washoensis* subsp. *cynomysii*, and strain 08S-0475 was supported by bootstrap analysis. The GenBank accession number for each sequence is shown in parentheses following each *Bartonella* strain. The dendrogram was rooted using the *gltA* sequence from *Brucella melitensis* strain 16M as an outgroup.

MicroScan Rapid Anaerobe identification panel (Siemens Healthcare Diagnostics, Inc., New Castle, DE). The profile of peptidase activity was similar to that described for *Bartonella* spp. and did not yield a profile unique to this isolate (14). Fatty acid methyl esters were prepared and analyzed using the Sherlock microbial identification system (MIDI, Newark, DE) and yielded major peaks of $C_{18:1w7t}$, $C_{18:0}$, and $C_{16:0}$, a feature consistent among most *Bartonella* spp.

To provide species-level resolution, PCR amplification and partial 16S rRNA, *groEL*, and *gltA* gene sequencing of strain 08S-0475 were performed using previously described primers (9, 11, 12). Amplification was not observed with the reagent control or negative extraction control for each of these PCR targets, eliminating the possibility of a false-positive result. Bidirectional sequencing of the 16S rRNA gene, *groEL*, and *gltA* PCR products resulted in 1,324 bp (GenBank accession number FJ719017), 538 bp (GenBank accession number FJ695137), and 336 bp (GenBank accession number FJ719016) of consensus sequence, respectively. A BLAST search of the National Center for Biotechnology Information's nucleotide database revealed 100% 16S rRNA gene sequence identity with "*Bartonella washoensis*" strain NVH1 isolated from a human source (GenBank accession number AF070463) and *B. washoensis* strain Sb944nv (GenBank accession number AB292597) isolated from a California ground squirrel (*Spermophilus beecheyi*). A similar search using the strain 08S-0475 *groEL* sequence resulted in 100% sequence identity with *B. washoensis* strain Sb944nv (GenBank accession number AF484066) and 99.6% sequence identity with a GenBank entry for the *B. washoensis* strain NVH1 (GenBank accession number AF071193). The strain 08S-0475 *gltA* sequence shared 100% sequence identity with *Bartonella* sp. strain Sb1659nv

(GenBank accession number AY071858) isolated from a California ground squirrel (*S. beecheyi*). The best species-level match was with several *B. washoensis gltA* sequences (including a *gltA* sequence derived from the human isolate, strain NVH1; GenBank accession number AF050108) with >98% sequence identity. The relationship of strain 08S-0475 *gltA* sequence to other *gltA* sequences derived from validated *Bartonella* species was evaluated using the neighbor-joining cluster analysis algorithm (Fig. 1). Bootstrap analysis revealed a statistically reliable cluster formed by strain 08S-0475, *B. washoensis* strain NVH1, and *B. washoensis* subsp. *cynomysii* strain CL8606co. This cluster was also supported using other phylogenetic algorithms, including maximum parsimony and maximum likelihood. Similarly, the strain 08S-0475 *groEL* sequence clustered with the *groEL* sequences from other *B. washoensis* strains compared by phylogenetic analysis (data not shown). Taken together, these results strongly support the identification of strain 08S-0475 as *B. washoensis*.

A field investigation of the patient's property was conducted approximately 3 months after disease onset to establish the role of ectoparasites in disease transmission. The patient resided on a rural 10-acre parcel located near the community of Browns Valley, California, which is part of the lower foothill metamorphic belt within the foothills section of the Sierra Nevada mountains. The property consisted of mostly open grass pasture intermixed with wooded areas. Numerous ground squirrel burrows and mounds were observed. Although seasonal cool weather suggested minimal rodent activity, trapping of rodents was attempted over a 24-hour period using 20 National live traps (National Live Trap Corp., Tomahawk, WI) and 100 Sherman live traps (Sherman Trap Company, Tallahassee, FL) deployed throughout the property and outbuildings. A single California ground squirrel (*S. beecheyi*) was captured. Fleas from this rodent were collected by combing and stored in 100% ethanol for later analysis. Additional sampling included flagging of ground squirrel burrows for fleas and collecting ticks from vegetation and resident dogs. Totals of 122 fleas (118 *Oropsylla montana* fleas, 3 *Hoplopsyllus anomalus* fleas, and 1 *Pulex simulans* flea) and 21 ticks (all *Ixodes pacificus*) were collected. Fleas were separated into pools of 1 to 16 fleas according to species and collection site for testing. Nucleic acids from flea pools and individual ticks were tested using *gltA* PCR and DNA sequencing. Amplification of *gltA* was not detected for any of the 21 *I. pacificus* ticks tested. However, *gltA* amplification and gene sequence was obtained for 8 of 11 flea pools (Table 1). Of the three flea species tested, only *O. montana* was positive for *gltA* amplification. Two distinct *gltA* sequences were detected and were found to match the sequences derived from either *B. washoensis* strain NVH1 or *Bartonella* sp. strain Sb1659nv. Sampling of the ground squirrel and burrow site 2 were found to contain fleas associated with both *gltA* sequences, suggesting that two *Bartonella* strains were present within these flea populations. These findings implicate *O. montana* fleas as the possible vector for bartonellosis in this case.

TABLE 1. *gltA* PCR and sequencing of fleas collected from the human patient's property

Flea source	Flea species	No. in test pool	*gltA* sequence ID[a]
Ground squirrel[b]	*O. montana*	16	*B. washoensis* strain NVH1
	O. montana	16	*B. washoensis* strain NVH1
	O. montana	13	*Bartonella* sp. strain Sb1659nv
	H. anomalas	3	Not detected
Ground squirrel burrows			
Site 1	*O. montana*	12	*B. washoensis* strain NVH1
Site 2	*O. montana*	16	Not detected
	O. montana	16	*Bartonella* sp. strain Sb1659nv
	O. montana	15	*B. washoensis* strain NVH1
Site 3	*O. montana*	5	*Bartonella* sp. strain Sb1659nv
Site 4	*O. montana*	9	*B. washoensis* strain NVH1
Flagging vegetation	*P. simulans*	1	Not detected

[a] 100% sequence identity (ID) with the indicated organism.
[b] *Spermophilus beecheyi*.

Members of the genus *Bartonella* are short, pleomorphic gram-negative bacilli that can adhere to and invade erythrocytes and endothelial cells. They are slow growing, hemin-dependent organisms that may take several weeks to produce detectable growth upon primary isolation. Laboratory identification of *Bartonella* species is difficult because of their fastidious nature and relative biochemical inactivity and is largely reliant upon gene sequence analyses (8). At least 10 species of the genus *Bartonella* have been associated with human disease, and several of these species are considered to be emerging zoonotic pathogens (8). Domestic cats represent a significant reservoir for the transmission of *Bartonella* infections to humans, particularly cat scratch disease (CSD) (7). Dogs have also been suggested as a possible reservoir of *Bartonella* (7). A much smaller disease burden has been associated with the rodent-borne *Bartonella* spp. which include *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis*, and *B. washoensis* (3). Human cases associated with rodent-borne *Bartonella* spp. have presented as febrile illnesses, endocarditis, myocarditis, and ocular manifestations, including neuroretinitis. The mode of disease transmission of these agents has not been well established but likely involves fleas or ticks as vectors.

This report presents the second case of a human infected with *B. washoensis*-like bacteria and the first detailed clinical description. The initial isolate, strain NVH1, originated from a 70-year-old man who presented with fever and myocarditis (11). The proposed species designation, *B. washoensis*, was based on phylogenetic comparison of nucleic acid sequences derived from this single *Bartonella* isolate with sequences from other recognized *Bartonella* species (4, 11). However, further information regarding the patient's clinical history or phenotypic characterization of the isolate has not been reported. The second human case, described here, presented with clinical signs and symptoms suggestive of meningitis (fever, headache, stiff neck, and photophobia) and possible low-grade sepsis (hypotension, pancytopenia). While analysis of CSF did not show significant leukocytosis or other abnormalities consistent with meningitis, the benign findings are consistent with other re-

ports of *Bartonella*-associated infections of the central nervous system. In a series of cases describing CSD encephalopathy due to *Bartonella henselae*, CSF findings were normal in over two-thirds of the 51 patients tested (2). As in the present case, most patients with CSD encephalopathy recover completely despite the severity of the initial clinical presentation. Encephalopathy is a rare complication of CSD; our findings suggest that *B. washoensis* may also be an infrequent cause of fever of unknown origin and central nervous system complications in patients with an exposure history that includes arthropod bites or contact with rodents.

An investigation of the first human case revealed that rodents within the vicinity of the patient's residence in Washoe County, Nevada, may have served as a reservoir for *B. washoensis*. Analyses of partial 16S rRNA, *gltA*, and *groEL* gene sequences of an isolate collected from a California ground squirrel (*S. beecheyi*) were identical to those of the isolate derived from the patient (11). In 2003, an isolate from a canine patient suffering from mitral valve endocarditis was identified as *B. washoensis* based on phenotypic, genetic, and serologic evidence (6). Both reports proposed that California ground squirrels and their fleas, especially *O. montana*, play an important role in the transmission of *B. washoensis*. Molecular studies have shown that *O. montana* fleas and *I. pacificus* ticks can harbor genetic sequences identical to those of *B. washoensis* and implicate these arthropods as potential vectors for transmission of this pathogen (5, 10, 13). Additional studies have found that bacterial isolates derived from black-footed prairie dogs (*Cynomys ludovicianus*) and their fleas (*O. montana* and *Oropsylla hirsute*) collected in Colorado are genetically similar to *B. washoensis* and have led to a proposed new subspecies, *B. washoensis* subsp. *cynomysii* (1).

The patient in the case described here had direct contact with rodents in the form of handling a dead mole and a dead ground squirrel. Ground squirrels have been suggested as the major reservoir for *B. washoensis* in California and Nevada (3, 6, 11). *Oropsylla montana* is the most common flea species to parasitize California ground squirrels. Our ability to match the *gltA* sequence from *O. montana* fleas collected from the patient's putative site of exposure with the *gltA* sequence from bacteria isolated from the patient strongly implicates these arthropods as a vector for disease transmission. Although the patient did not recall any recent arthropod bites, it is possible that she was bitten by an infected flea while handling the squirrel carcass or while walking near ground squirrel burrows on her property. Our findings provide additional support for the potential role of *O. montana* fleas as the vector and *S. beecheyi* ground squirrels as the reservoir for *B. washoensis* infections—a mode of transmission that parallels that of plague (*Yersinia pestis*) in California.

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