JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2003, p. 5327–5332 Vol. 41, No. 11
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.11.5327–5332.2003

Isolation of *Bartonella washoensis* from a Dog with Mitral Valve Endocarditis

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Received 7 March 2003/Returned for modification 22 June 2003/Accepted 11 August 2003

We report the first documented case of *Bartonella washoensis* bacteremia in a dog with mitral valve endocarditis. *B. washoensis* was isolated in 1995 from a human patient with cardiac disease. The main reservoir species appears to be ground squirrels (*Spermophilus beecheyi*) in the western United States. Based on echocardiographic findings, a diagnosis of infective vegetative valvular mitral endocarditis was made in a spayed 12-year-old female Doberman pinscher. A year prior to presentation, the referring veterinarian had detected a heart murmur, which led to progressive dyspnea and a diagnosis of congestive heart failure the week before examination. One month after initial presentation, symptoms worsened. An emergency therapy for congestive heart failure was unsuccessfully implemented, and necropsy evaluation of the dog was not permitted. Indirect immunofluorescence tests showed that the dog was strongly seropositive (titer of 1:4,096) for several *Bartonella* antigens (*B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, and *B. henselae*), highly suggestive of *Bartonella* endocarditis. Standard aerobic and aerobic-anaerobic cultures were negative. However, a specific blood culture for *Bartonella* isolation grew a fastidious, gram-negative organism 7 days after being plated. Phenotypic and genotypic characterizations of the isolate, including partial sequencing of the citrate synthase (*gltA*), *groEL*, and 16S rRNA genes, indicated that this organism was identical to *B. washoensis*. The dog was seronegative for all tick-borne pathogens tested (*Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Rickettsia rickettsii*), but the sample was highly positive for *B. washoensis* (titer of 1:8,192) and, according to indirect immunofluorescent-antibody assay, weakly positive for phase II *Coxiella burnetii* infection.

CASE REPORT

On 2 August 2001, a spayed 12-year-old female mixed Doberman pinscher dog weighing 30 kg presented to the Veterinary Centers of America Emergency Animal Hospital and Referral Center for evaluation of a murmur by a board-certified cardiologist. One year prior to presentation, the referring veterinarian had detected a heart murmur and placed the patient on a treatment regimen of 0.33 mg of enalapril/kg of body weight per os (p.o.) twice daily (BID). One week prior to referral, the patient had been evaluated for progressive dyspnea. Thoracic radiographs performed at that time revealed moderate cardiomegaly and severe enlargement of the left atrium, as well as a mild perihilar pulmonary interstitial pattern. A solitary pulmonary nodule, approximately 2 cm in diameter, was also visible within the left caudal lung lobe. Based on thoracic radiographic changes, a presumptive diagnosis of congestive heart failure was made, and the patient was placed on a regimen of 1.67 mg of furosemide/kg p.o. BID. Other diagnostics performed at that time included a serum chemistry profile, a complete blood count, and a measurement of the serum thyroxine concentration. The diagnostics were unremarkable with the exception of mild monocytopenia (990 monocytes/μl; normal range, 1,000 to 4,800/μl) and mild hyperchloridemia (116 meq/liter; normal range, 105 to 115 meq/liter).

The history for the patient included intermittent incontinence that was managed with diethylstilbestrol twice weekly and degenerative joint disease that was treated with intermittent therapy with various nonsteroidal anti-inflammatory agents. Therapy with levothyroxine (0.02 mg/kg p.o. BID) had been carried out for 2 years for hypothyroidism. At 3 years of age, the patient underwent bilateral surgical correction of ruptured cranial cruciate ligaments. The patient also had a previous history of exposure to ticks and fleas.

On presentation to the cardiologist (A. C. Wey), the patient was tachycardic (160 beats/min) but normothermic (39°C, 102.2°F) with a normal respiratory rate of 28 breaths/min. Physical examination revealed multiple soft, small (<1-cm) cutaneous nodules consistent with lipomas. On thoracic auscultation, a grade IV to VI systolic murmur in the left apex was present, as well as an S3 gallop sound and an occasional arrhythmia with pulse deficits. The patient was slow to rise and appeared stiff in the hind limbs when ambulatory, but no joint swellings were noted. Electrocardiography revealed a heart rate of 120 beats/min, sinus arrhythmia with occasional premature atrial and ventricular contractions, and a normal mean electrical axis (60 degrees; normal range, 40 to 100). Echocardiography revealed moderate eccentric left-ventricular dilation (left-ventricular end diastolic diameter, 4.7 cm), severe left atrial enlargement (left atrium, 4.5 cm; left atrium/aorta, 2.0 cm), and severe mitral regurgitation (velocity decreased at 4.2 m/s). A large, hyperechoic mass lesion was noted adherent to the anterior mitral valve leaflet that prolapsed into the left atrium during systole. The anatomy of all other cardiac valves appeared normal, and no other valvular insufficiencies were noted. Based on the echocardiographic findings, a presumptive

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diagnosis of vegetative endocarditis of the mitral valve was made. Further possible diagnostics discussed with the owner included spinal radiographs, abdominal ultrasound, urine and blood cultures, various serological tests, and joint taps. Only serology for the presence of *Bartonella* sp. antibodies and a blood culture to attempt *Bartonella* isolation were performed at that time. However, because the dog had a history of tick and flea exposure, we later performed various serological tests. Therapy initiated at that time included amoxicillin-clavulanic acid (16.7 mg/kg p.o. BID), enrofloxacin (4.5 mg/kg p.o. BID), and an increase in the dosage of furosemide (3.3 mg/kg p.o. BID).

After 3 days, urine cultures were negative. Serial blood cultures obtained 30 min apart by a sterile technique from three different sites were negative for bacterial pathogens after 1 week. However, the dog was reported to have a high titer of antibodies to various *Bartonella* species, and the *Bartonella*-specific blood culture yielded a positive culture after 1 week (see below). At this time, therapy with amoxicillin-clavulanic acid was discontinued, and therapy with doxycycline (10 mg/kg p.o. BID) was initiated.

One month after initial presentation to the referred cardiologist, the patient was presented to another emergency animal hospital for treatment of progressive lethargy, anorexia, and dyspnea. Thoracic radiographs at that time revealed a severe perihilar pulmonary interstitial-to-alveolar pattern and persistent cardiomegaly (left-atrial enlargement). Emergency therapy for congestive heart failure, including intravenous diuresis, oxygen supplementation, and a topical venodilator, were unsuccessful, and the patient experienced cardiopulmonary arrest. Cardiopulmonary resuscitation was not attempted. Unfortunately, necropsy evaluation of the patient was not permitted.

Bartonella species are emerging pathogens in humans, causing severe diseases in immunocompromised individuals (10). At least eight *Bartonella* species or subspecies are known to be pathogenic for humans, including *B. bacilliformis*, *B. quintana*, *B. henselae*, *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis* (1, 18, 27, 47), *B. vinsonii* subsp. *berkhoffii* (44), and *B. washoensis* (10, 32). Furthermore, based on serological evidence, *B. clarridgeiae* has been associated with cat scratch disease in humans (29, 35, 45). Among these *Bartonella* species or subspecies, mainly *B. quintana* and *B. henselae* (1, 7, 10, 19, 41, 42, 46) but also *B. elizabethae* and *B. vinsonii* subsp. *berkhoffii* have been identified as causative agents of human endocarditis (18, 44), and *B. washoensis* has been identified as a cause of myocarditis (10, 32). The main reservoir species for *B. washoensis* appears to be ground squirrels (*Spermophilus beecheyi*) in Nevada (32) and California (B. B. Chomel, C. C. Chang, A. C. Ziedins, R. W. Kasten, and C. M. Hew, Abstr. 55th Int. Northwest. Conf. Dis. Nat. Commun. Man, 30 July to 2 August, 2000).

The number of *Bartonella* species or subspecies causing clinical diseases in dogs has been increasing in recent years. *B. vinsonii* subsp. *berkhoffii* has been shown to cause endocarditis, arrhythmia, and myocarditis (8, 11, 30), as well as granulomatous lymphadenitis and granulomatous rhinitis (39). *B. clarridgeiae* was isolated from the blood of a dog suffering from aortic endocarditis (17). *B. clarridgeiae* DNA was also detected by PCR in a dog with lymphocytic hepatitis (22). *B. henselae* DNA was initially detected in a dog with peliosis hepatis (28) and more recently in a dog with hepatopathy (22) and in three dogs with various clinical entities (37). These three *B. henselae*-PCR-positive dogs presented nonspecific clinical abnormalities, such as severe weight loss, protracted lethargy, and anorexia. A fourth dog was diagnosed as being infected with *B. elizabethae* by PCR amplification and sequencing, increasing the number of *Bartonella* species identified in infected dogs (37). We report the first isolation of another *Bartonella* species, *B. washoensis*, from the blood of a dog suffering from mitral valve vegetative endocarditis.

Strain sources. The type strains of *B. vinsonii* subsp. *berkhoffii* (ATCC 51672) and *B. clarridgeiae* (ATCC 51734) were obtained from the American Type Culture Collection (Manassas, Va.). Isolate *B. henselae* strain U4 was obtained from our culture collection at the University of California, Davis. Isolate UCD-dog2 was cultured from the blood of the dog described in this report.

Clinical samples. Blood (6 ml) was collected aseptically from the dog's external jugular vein at the time of clinical examination by A. C. Wey. Blood samples were subjected to aerobic and aerobic-anaerobic cultures. A blood sample, collected in a plastic EDTA tube (Becton Dickinson, Franklin Lakes, N.J.), was also submitted for specific *Bartonella* isolation, and the blood was plated on fresh blood agar (5% defibrinated rabbit blood) (15). Serum was submitted for *Bartonella* antibody titers and was later tested for several tick-borne pathogens (*Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Rickettsia rickettsii*), including *Coxiella burnetii*, a known agent of endocarditis in humans and animals.

Isolation procedure. A blood sample was collected in a plastic 2-ml EDTA tube and frozen at −70°C until plated. The blood sample was cultured on heart infusion agar containing 5% rabbit blood and incubated in 5% CO_2 at 35°C for up to 4 weeks. Identification of the isolates as *Bartonella* spp. was initially based on morphological and growth characteristics, as previously described (17, 24). The isolates were then confirmed by PCR and restriction fragment length polymorphism (RFLP) analysis and sequence analysis of the citrate synthase (*gltA*), *groEL*, and 16S rRNA genes, as previously described (23, 36, 38).

Microscopic and biochemical analysis. DNA extraction. A loopful (10 μl) of the bacterial isolate grown from the dog's blood was heated at 95°C for 10 min in 100 μl of sterile water. The supernatant was used as a DNA template, as previously described (12, 17).

PCR assay. Five microliters of extracted DNA from the bacterial isolate was added to a 45-μl reaction mixture consisting of 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, a 200 μM concentration of each deoxynucleoside triphosphate, 1.25 U of AmpliTaq Gold DNA polymerase (Perkin Elmer, Foster City, Calif.), and a 0.25 μM concentration of each primer. The primers used for the *gltA* gene were 5′-GGGGA CCAGCTCATGGTGG-3′ and 5′-AATGCAAAAAGAACA GTAAACA-3′ (38). Thermocycling was performed in an MJ Research PTC-100 (Watertown, Mass.) apparatus under the following conditions: 94°C for 10 min and 45 cycles of 94°C for

0.5 min, 57°C for 1 min, and 72°C for 2 min, followed by 72°C for 5 min. For the 16S rRNA gene, the primers were 5′-AGA GTTTGATCCTGGCTCAG-3′ and 5′-AAGGAGGTGATCC AGCCGCA-3′ (23), and the running cycles were as follows: 95°C for 10 min and 35 cycles of 93°C for 0.5 min, 60°C for 1 min, and 72°C for 1 min, followed by 72°C for 8 min. For the *groEL* gene, the primers were 5′-CGTGAAGTTGCCTCAAA AACC-3′ and 5′-AATCCATTCCGCCCATTC-3′ (36), and the running cycles were as follows: 95°C for 10 min and 40 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 1 min, followed by 72°C for 5 min.

RFLP analysis. An approximately 400-bp fragment amplified from the *gltA* gene was verified by gel electrophoresis and then digested with the *Taq*I (Promega, Madison, Wis.), *Hha*I, *Aci*I, and *Mse*I (New England Laboratories, Beverly, Mass.) restriction endonucleases (5, 43). The digestion conditions used were the ones recommended by the enzymes' manufacturers. Banding patterns of the digests were compared to those of digests from *B. vinsonii* subsp. *berkhoffii* (ATCC 51672), *B. henselae* (strain U-4; University of California, Davis), and *B. clarridgeiae* (ATCC 51734).

DNA sequencing. The PCR products from the *gltA*, 16S rRNA, and *groEL* genes used for DNA sequencing were purified with Microcon centrifugal filter devices (Millipore Corp., Bedford, Mass.) and sequenced with a fluorescence-based automated sequencing system (Davis Sequencing, Davis, Calif.), as previously described (14, 36). The FastA program of SeqWeb version 2 (Wisconsin Package; Accelrys Inc., San Diego, Calif.) was applied first to search the GenBank and EMBL databases for the closest bacterial species or subspecies. Then, the GAP and Evolution programs were used for sequence alignments and determination of the percentage of DNA similarity.

IFA tests. (i) *Bartonella* spp. *B. henselae*, *B. clarridgeiae*, *B. vinsonii* subsp. *berkhoffii*, and *B. washoensis* indirect immunofluorescent-antibody (IFA) tests were performed as previously described (14–16). The intensity of bacillus-specific fluorescence was scored subjectively from 1 to 4, and a fluorescence score of ≥2 at a dilution of 1:64 was reported as a positive result, as previously described (14–16). Except for *B. washoensis*, the same two readers performed a double-blind reading of each slide. Positive serum control samples were obtained from dogs that had been confirmed to be infected with *Bartonella*. Specific IFA serology for *B. washoensis* was performed at the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colo.

(ii) *E. canis*, *A. phagocytophilum*, *R. rickettsii*, and *C. burnetii*. An IFA test was used to detect antibodies to *E. canis* (North Carolina State University canine strain), *A. phagocytophilum* (New York strain, human origin), and *R. rickettsii* (Domino canine strain) on 30-well Teflon-coated slides (9, 40). Serial twofold dilutions of the dog's serum were reacted with fluorescein isothiocyanate anti-canine immunoglobulin G conjugate (Cappel; ICN Pharmaceuticals, Inc., Costa Mesa, Calif.). Endpoint titers were determined as the last dilution at which brightly stained organisms could be detected on a fluorescence microscope with exciter and barrier filters. Antibodies against phase I and phase II of *C. burnetii* infection were evaluated with a commercially available IFA substrate slide (Fuller Laboratories, Fullerton, Calif.) at a 1:64 dilution (17).

Isolation and identification of *B. washoensis*. Standard blood cultures for aerobic and anaerobic bacteria failed to grow bacteria. However, blood collected from the dog at the same time and cultured from the frozen and thawed EDTA tube grew a nonhemolytic gram-negative organism after 7 days, with only a few (60 CFU per ml) small (0.5- to 1-mm) white colonies. These colony growth characteristics and morphologies were suggestive of *Bartonella* spp., based on size, color, and time for colonies to appear. Microscopic examination demonstrated small gram-negative rods. PCR amplification with *gltA* primers produced a 400-bp fragment strongly suggestive of *Bartonella*. Digestion with *Taq*I, *Hha*I, *Aci*I, and *Mse*I endonucleases produced a unique RFLP profile.

A search (FastA) of the GenBank and EMBL databases with the *gltA* fragment (352 bp) resulted in the closest matches to *B. washoensis* (accession no. AF050108) and *Bartonella* sp. strain Sb944nv (AF470616) isolated from a California ground squirrel, with *z* scores of 1,334.8 and 1,330.9, respectively, where *z* is an indication of relatedness. A direct comparison of the dog isolate to *B. washoensis* (AF050108) with the GAP program yielded a 100% identity. Comparison of this sequence with other *Bartonella gltA* sequences (Evolution/Grow Tree program) resulted in a cluster containing the dog's isolate, *B. washoensis*, and the California ground squirrel *Bartonella* sp. strain Sb944nv (AF470616) isolate with identical sequences. Numerous other strains isolated from Nevada rodents (ground squirrels, least chipmunks, and bushytail woodrats) were also closely related to the dog isolate, with a divergence of 1.94 to 3.59 base substitutions per 100 bases (Table 1). A group of isolates cultured from deer mice (*Peromyscus maniculatus*) appear to be more divergent, with values of 7.01 to 7.68 base substitutions per 100 bases. Divergences of other standard *Bartonella* strains were 6.98 (*B. vinsonii* subsp. *arupensis*) to greater than or equal to 7.68 (*B. henselae*) base substitutions per 100 bases. Similarly, a fragment of the *groEL* gene (306 bp) of the dog isolate was compared to *Bartonella* strains listed in the GenBank and EMBL databases. The dog isolate was identical to *B. washoensis* and *Bartonella* sp. strain Sb944nv (AF484066) from a ground squirrel and similar to *Bartonella* sp. strain Sb1963nv (AF484067) from another California ground squirrel, with a base substitution rate of 0.33 per 100 bases compared to at least 6.82 per 100 bases for other *Bartonella* strains (Table 2). Finally, when a fragment of the 16S rRNA gene (511 bp) was compared, the dog isolate was 100% identical to *B. washoensis* (AF070463). A slightly longer fragment (577 bp) indicated a minor divergence, with 0.17 base substitutions per 100 bases, although the 16S rRNA gene comparison indicated a variance of less than 3 base substitutions per 100 bases for the group analyzed.

Serological analysis. Serum antibodies to *B. clarridgeiae*, *B. vinsonii* subsp. *berkhoffii*, and *B. henselae* were detected in the dog by IFA assay at the reciprocal titer of 4,096. Because of the dog's history of being exposed to fleas and ticks, the dog serum was later tested for potential tick-borne pathogens. The dog was seronegative for *A. phagocytophilum*, *E. canis*, and *R. rickettsii* but was weakly positive for the *C. burnetii* phase II antigen. After identification of the isolate as *B. washoensis*, the dog

TABLE 1. Estimated number of nucleotide substitutions per 100 bases for a 315-base segment of the *gltA* gene

Strain and/or species	GenBank accession no.	Rate of divergent base substitutions
UCD-dog2		0
B. washoensis	AF050108	0
Bartonella sp. (Sb944nv)	AF470616	0
Bartonella sp. (Sb1659nv)	AY071858	1.94
Bartonella sp. (Al7763nv)	AF071188	2.58
Bartonella sp. (Al7760nv)	AF071187	2.91
Bartonella sp. (S1311nv)	AY071861	2.92
Bartonella sp. (Sb1865nv)	AY071860	2.92
Bartonella sp. (Tm1950nv)	AF451161	2.92
Bartonella sp. (Tm918nv)	AF451159	3.24
Bartonella sp. (Tm916nv)	AF451160	3.24
Bartonella sp. (Sb1859nv)	AY071859	3.25
Bartonella sp. (Tm1781nv)	AF451162	3.25
Bartonella sp. (Tm6313nv)	AF071189	3.58
Bartonella sp. (Tm1794nv)	AF451163	3.59
B. vinsonii subsp. *arupensis*	AF214557	6.98
Bartonella sp. (Pm1785nv)	AY064535	7.01
Bartonella sp. (Pm1780nv)	AY064533	7.33
Bartonella sp. (Pm1783nv)	AY064534	7.36
Bartonella sp. (Pm1857nv)	AY064536	7.68
B. henselae	BAOGLTA	7.68
B. koehlerae	AF176091	8.39
B. vinsonii subsp. *berkhoffii*	BVU28075	8.74
B. quintana	BQCSFULLER	9.10
B. vinsonii subsp. *vinsonii*	BVCSBAKER	9.46
B. birtlesii	AF204272	9.46
B. taylorii	BTCSM6	10.55
B. tribocorum	BTA005494	10.92
B. alsatica	AF204273	11.65
B. grahamii	BGCSV2	11.65
B. bovis	AF293394	11.65
B. elizabethae	BEU28072	12.40
B. capreoli	AF293392	13.15
B. schoenbuchensis	AJ278186	13.15
B. doshiae	BDCSR18	13.53
B. clarridgeiae	BCU84386	13.53
B. bacilliformis	BBU28076	18.24

serum was tested against this specific antigen and revealed a reciprocal titer of 8,192.

Conclusions. We report the first isolation of *B. washoensis* from a dog and the second isolation of this species outside of its natural rodent reservoir. It is also the first known case of endocarditis associated with *B. washoensis*, as it caused fever and myocarditis in the human patient (32). In this dog, several factors are suggestive that *B. washoensis* was the etiological agent, including (i) isolation of the organism from the blood, (ii) failure to isolate other bacteria by conventional blood culture, and (iii) the presence of high titers of antibody against various *Bartonella* species, including *B. washoensis*, as previously reported for cases of *Bartonella* endocarditis in humans (21, 42) and dogs (K. A. MacDonald, B. B. Chomel, M. D. Kittleson, R. W. Kasten, W. P. Thomas, and P. A. Pesavento. Abstr. Am. Coll. Vet. Intern. Med., 21st Annu. Forum, J. Vet. Intern. Med. **17:**399, 2003). Unfortunately, tissues were not available for microscopic examination or PCR testing of the damaged valve.

The valvular lesion was located on the mitral valve, as previously reported for other *Bartonella* endocarditis cases in dogs (8). However, most *Bartonella* endocarditis cases in dogs usually involve the aortic valve or both cardiac valves (8, 11; MacDonald et al., Abstr. Am. Coll. Vet. Intern. Med.). Similarly, in a series of 33 human patients with *Bartonella* endocarditis, 29 (88%) had involvement of the aortic valve, including 2 with concurrent aortic and mitral valvular involvement, compared to only 4 patients with only involvement of the mitral valve (41). In a more recent study of 61 confirmed cases of *Bartonella* endocarditis, involvement of only the aortic valve (36 cases) was four times more frequent than involvement of only the mitral valve (9 cases) (42).

Genetic analysis of the dog isolate indicates that the organism isolated is *B. washoensis*, as the sequence analysis of fragments from three different genes revealed genetic identity with *B. washoensis* isolated from a human patient with myocarditis and a California ground squirrel (32). Furthermore, the dog isolate was clustered with several other rodent isolates cultured from animals captured in the vicinity of the human patient. We also found a complete match between the human and dog isolates for the *groEL* gene partial sequence and a minor mismatch with isolate Sb1963nv, as reported by Kosoy et al. (32).

Analysis of the *gltA* gene has historically been used to compare *Bartonella* strains since it provides a good discriminatory power at the species level (5, 38). While analysis of the 16S rRNA gene provides usable information at the genus level, the high degree of similarity among *Bartonella* strains disallows strong discriminatory power for many of the new species of *Bartonella* (5, 26). Sequences from other genes, such as *groEL*, are beginning to be published and provide an opportunity to compare more fragments from each isolate.

The dog's source of infection and the mode of transmission of *B. washoensis* will most likely remain unexplained. We know that the dog had a history of tick and flea exposure. However, this dog was seronegative for associated bacteria, in contrast to dogs infected with *B. vinsonii* subsp. *berkhoffii*, for which a strong association has been reported between seropositivity to *Bartonella* and seropositivity to several tick-borne pathogens, especially *Ehrlichia*, *Anaplasma*, and *Babesia* spp. (9, 31, 40). Furthermore, the weak positive reaction to the *C. burnetii*

TABLE 2. Estimated number of nucleotide substitutions per 100 bases for a 306-base segment of the *groEL* gene

Strain and/or species	GenBank accession no.	Rate of divergent base substitutions
UCD-dog2		0
B. washoensis	AF071193	0
Bartonella sp. (Sb944nv)	AF484066	0
Bartonella sp. (Sb1963nv)	AF484067	0.33
B. vinsonii subsp. *arupensis*	AF30416	6.82
B. doshiae	AF014832	8.26
B. vinsonii subsp. *berkhoffii*	AF014836	8.62
B. grahamii	AF014833	8.62
B. taylorii	AF304017	8.62
B. vinsonii subsp. *vinsonii*	AF014835	9.35
B. henselae	AF014829	9.72
B. birtlesii	AF355773	10.47
B. alsatica	AF299357	10.85
B. quintana	AF014830	10.85
B. tribocorum	AF304018	11.98
B. bacilliformis	BAOBB63A	13.53
B. elizabethae	AF014834	15.10
B. clarridgeiae	AF014831	16.31

phase II antigen is more likely to result from serological cross-reactivity, as was previously demonstrated for humans (34). *B. washoensis* has been reported to have a rodent reservoir (10, 32). In California and Nevada, a high percentage of California ground squirrels are infected with this species and represent its main reservoir (32; B. B. Chomel et al. Abstr. 55th Int. Northwest. Conf. Dis. Nat. Commun. Man).

However, the arthropod vector of *B. washoensis* has not yet been identified. The ground squirrels from northern California, among which *B. washoensis* bacteremic animals were identified, were heavily infested with fleas (Chomel et al., unpublished observations). As indicated by Kosoy et al. (32), *S. beecheyi* are commonly and heavily infested with *Oropsylla montana*, a flea species that readily feeds on humans and generally is considered to be the most important vector of human plague in the United States. These fleas might play a main role as the vector of infection between rodents and also might be involved in the transmission of *B. washoensis* to dogs and humans. However, the potential role of ticks still cannot be excluded, as several questing adult *Ixodes pacificus* ticks have also been reported to harbor *B. washoensis* DNA (13).

Rodents constitute a wide reservoir of *Bartonella* species in many parts of the world (2, 3, 4, 6, 10, 20, 24, 25, 33, 48). In North America, several *Bartonella* species or subspecies have been identified, including *B. vinsonii*, *B. vinsonii* subsp. *arupensis*, *B. elizabethae*, *B. peromysci*, and *B. washoensis* (6, 10, 20, 25, 33, 47), and many more are still waiting to be fully described (4, 20, 33, 48). Among these rodent-borne *Bartonella* spp., four species (*B. elizabethae*, *B. vinsonii* subsp. *arupensis*, *B. grahamii*, and *B. washoensis*) have now been related to human infections, causing mainly cardiopathy, endocarditis, or neuroretinitis (10, 32). Furthermore, *B. elizabethae* DNA was recently identified in a sick dog with a 2-month history of lethargy, decreased appetite, weight loss, and occasional vomiting of undigested food (37). With the present report, it is the second rodent-borne *Bartonella* species to be found in sick dogs, suggesting that many other *Bartonella* species may cause severe infections in domestic dogs.

This project was supported in part by a grant from the Morris Animal Foundation and a grant from the Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis.

We thank Barbara Hegarty and Edward Breitschwerdt, College of Veterinary Medicine, North Carolina State University, for testing the dog serum for the various tick-borne pathogens and Michael Kosoy, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, for providing assistance for the *B. washoensis* IFA testing.

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