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Molecular Characterization of *Bartonella vinsonii* subsp. *berkhoffii* Genotype III▽

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The molecular characterization of a *Bartonella vinsonii* subsp. *berkhoffii* genotype III strain (NCSU strain 06-CO1) isolated from the blood of a military working dog diagnosed with endocarditis is reported in this study. Several genes were amplified and sequenced for comparative sequence similarity with other strains.

Bartonellae are fastidious hemotropic gram-negative bacteria which have been recently identified in a wide range of domestic and wild animals (2, 6). Based upon sequence differences within the 16S-23S intergenic spacer region (ITS) and the bacteriophage-associated heme-binding protein Pap31 gene (*pap31*), four *Bartonella vinsonii* subsp. *berkhoffii* genotypes have been characterized (3, 5, 11, 14). *B. vinsonii* subsp. *berkhoffii* genotype I has been isolated from dog, coyote, and human blood and from dog saliva (1, 3, 5, 10, 14); genotype II has been isolated from dog, coyote, and human blood and dog saliva (3, 10, 14); genotype III was isolated from gray foxes in the United States and was sequenced from a human endocarditis patient in Europe (14, 17); and genotype IV has only been amplified from two dogs with endocarditis, one from Canada and one from Colorado (7, 14; E. B. Breitschwerdt and R. G. Maggi, unpublished data). In this study, we report the molecular characterization of a *B. vinsonii* subsp. *berkhoffii* genotype III strain which was obtained by blood culture from a military working dog that originated from Germany and developed endocarditis while stationed in the United States.

Case report. A 3-year-old, 28.6-kg, spayed female German shepherd obtained from a breeder in Germany at 18 months of age was subsequently stationed in Texas. At procurement, the dog was healthy and serologically negative for *Dirofilaria immitis*, *Babesia canis*, *Ehrlichia canis*, *Borrelia burgdorferi*, and *Rickettsia rickettsii*. No clinical abnormalities were noted at her semiannual examination; however, a complete blood count revealed thrombocytopenia (platelet count, 168,000/μl; reference range, 200,000 to 500,000/μl). Three months later the left hock became significantly swollen, with mild crepitus and possible joint effusion. Overnight, the right hock also became painful and swollen; the dog was obtunded, febrile (rectal temperature 103.4°F), and had enlarged popliteal lymph nodes and bilateral pitting edema distal to the tarsus. Lymph node cytology identified lymphadenitis and plasmacytosis, with no etiologic agents visualized. The dog was thrombocytopenic (platelet count, 105,000/μl) and hyperglobulinemic (globulin, 4.7 g/dl). Ehrlichiosis was suspected, and doxycycline was administered (6.7 mg/kg of body weight every 12 h for 3 weeks). Within 9 days, the dog was clinically normal, with complete resolution of the hind-limb swelling and lameness; however, thrombocytopenia persisted on serial hemograms (range, 53,000 to 176,000 platelets/μl). Subsequently, by immunofluorescent antibody testing at the Vector Borne Diseases Diagnostic Laboratory at North Carolina State University, the reciprocal antibody titers were 64 for *Babesia canis*, 2,048 for *Bartonella henselae*, 8,192 for *B. vinsonii* subsp. *berkhoffii*, 64 for *Ehrlichia canis*, and 32 for *Rickettsia rickettsii*, and *Bartonella vinsonii* subsp. *berkhoffii* was isolated by *Bartonella-Alphaproteobacteria* growth medium (BAPGM) blood culture (13). Echocardiography identified marked thickening and irregularity of the aortic valve. Because of the poor prognosis associated with *Bartonella* endocarditis, the dog was humanely eutha-

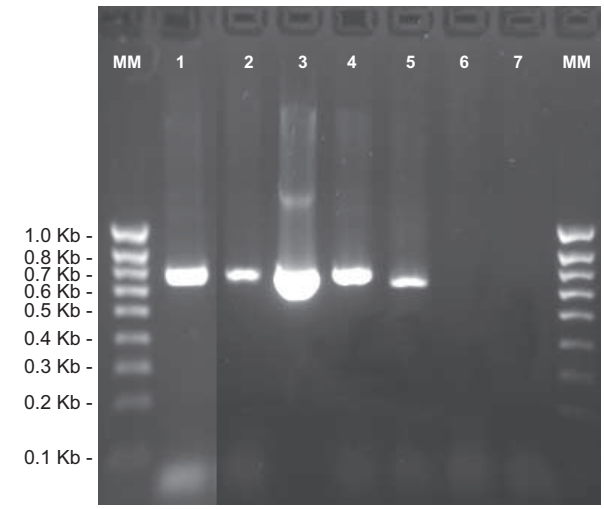

FIG. 1. *Bartonella* genus ITS PCR from blood, aortic valve, and left ventricular myocardium. ITS PCR amplicon products were resolved through 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. Lane 1, preantibiotic blood sample; lane 2, postantibiotic blood sample; lane 3, aortic valve; lane 4, left ventricle; lane 5, *Bartonella henselae* at 0.001 pg/μl; lane 6, blood from a healthy dog; lane 7, water control. MM, 1-kb molecular marker ladder.

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TABLE 1. List of primers used in PCRs

Region or gene name[a]	Primer name	Primer sequence	Reference
16S rRNA	8 f	5′-AGAGTTTGATCCTGGCTCAG-3′	20
	325 r	5′-CGCCAGAAGGCTTGGGATCATCATCTGAAG-3′	17
ITS	325 f	5′-TTCAGATGATGATCCCAAGCCTTTTGGCG-3′	17
	1100 r	5′-GAACCGACGACCCCCTGCTTGCAAAGCA-3′	17
pap31	1 f	5′-GACTTCTGTTATCGCTTTGATTT-3′	16
	688 r	5′-CACCACCAGCAAMATAAGGCAT-3′	16
rpoB	1615 f	5′-GACTTCTGTTATCGCTTTGATTT-3′	9
	2267 r	5′-CACCACCAGCAAMATAAGGCAT-3′	9
gdh1	8 f	5′-GTAAAATTATTCCAGTCTCTCCCTTT-3′	
	408 f	5′-TGTCCCAATCTCATCGCAATATCACC-3′	
ialB	49 f	5′-TTGAGTATTTCYTCTGYRTTTGC-3′	
	530 r	5′-TGCAAAGMARTYAAACGMTTAAGWGC-3′	

[a] *gdh1* and *ialB* primers were designed using *Bartonella quintana* and *B. henselae* glucose-6-phosphate 1-dehydrogenase and invasion-associated protein gene sequences from complete genomes in GenBank (accession no. BX897700 and BX897699, respectively).

nized. Histopathology confirmed aortic-valve endocarditis, with intralesional gram-negative coccobacilli, diffuse neutrophilic and histiocytic inflammation accompanied by hemorrhage, mineralization, marked granulation tissue, and fibrosis of the aortic valve. There was also multifocal lymphocytic plasmacytic myocarditis.

A blood culture isolate (NCSU strain 06-CO1) was obtained after BAPGM preenrichment culture and subinoculation onto blood agar plates (9, 13). Using *Bartonella* genus ITS primers, amplicons were obtained from blood, tissues, and the isolate (Fig. 1) (15). After cloning, DNA sequences differed by only one base among the various sample sources (14, 15). Based upon alignment of GenBank *Bartonella* ITS sequences, the dog was infected with *B. vinsonii* subsp. *berkhoffii* genotype III. As this was the first *B. vinsonii* subsp. *berkhoffii* genotype III isolate from the United States, the partial 16S rRNA gene (4, 15, 18), 16S-23S ITS region (14), *pap31* gene (14), RNA polymerase enzyme subunit B gene (*rpoB*) (8), glucose-6-phosphate 1-dehydrogenase gene (*gdh1*), and invasion-associated protein B gene (*ialB*) were sequenced and compared to GenBank sequences using BLAST. Primers, alignment results, and sequence comparisons are shown in Tables 1 and 2. NCSU strain 06-CO1 had higher percent identities with two *B. vinsonii* subsp. *berkhoffii* ITS sequences recently deposited from dog

TABLE 2. *Bartonella vinsonii* subsp. *berkhoffii* genotype III (NCSU strain 06-CO1) partial gene sequence identities with other *Bartonella* strain sequences

Region or gene	% Identity	GenBank accession no.	Organism designation	Origin
ITS region	99.9	AF143446	*B. vinsonii* subsp. *berkhoffii* type III	Amplified from a human aortic valve
	99.7	DQ360834	*B. vinsonii* subsp. *berkhoffii* type III strain Q52SHD	Blood culture isolate from a dog
	99.7	DQ360833	*B. vinsonii* subsp. *berkhoffii* type III strain Q64SHD	Blood culture isolate from a dog
	98.9	DQ059764	*B. vinsonii* subsp. *berkhoffii* type III	Blood culture isolate from a gray fox
pap31	99.3	DQ059762	*B. vinsonii* subsp. *berkhoffii* type II	Blood culture isolate from a dog
	99.1	AY663045	*B. vinsonii* subsp. *berkhoffii* type I 93-COI	Blood culture isolate from a dog
	98.7	DQ112677	*B. vinsonii* subsp. *berkhoffii* type IV	Amplified from a dog's aortic valve
	98.4	DQ071677	*B. vinsonii* subsp. *berkhoffii* type III	Blood culture isolate from a gray fox
16S rRNA	100	DQ228134	*B. vinsonii* subsp. *berkhoffii* type III strain Q52SHD	Blood culture isolate from a dog
	100	DQ228135	*B. vinsonii* subsp. *berkhoffii* type III strain Q64SHD	Blood culture isolate from a dog
	100	AF143446	*B. vinsonii* subsp. *berkhoffii* type III	Amplified from a human aortic valve
	99.7	L35052	*B. vinsonii* subsp. *berkhoffii* type I strain 93-COI	Blood culture isolate from a dog
	99.7	none	*B. vinsonii* subsp. *berkhoffii* type II	Blood culture isolate from a dog
rpoB	99.8	AF165989	*B. vinsonii* subsp. *berkhoffii* type I strain 93-COI	Blood culture isolate from a dog
	99.8	EF196805	*B. vinsonii* subsp. *berkhoffii* type I by ITS	Blood culture isolate from a dog
	99.8	none	*B. vinsonii* subsp. *berkhoffii* type II by ITS	Blood culture isolate from a dog
gdh1	87	AY074765	*B. henselae* strain Houston 1	Database of complete genome
	87	BX897699	*B. henselae* strain Houston 1	Complete genome from a human isolate
ialB	88	BX897700	*Bartonella quintana* strain Toulouse	Complete genome from a human isolate

blood culture isolates (GenBank accession no. DQ360834 and DQ360835) obtained in China (12) and a sequence from a human endocarditis case from Europe (GenBank accession no. AF143446) (17) and a less-similar identity to a gray fox sequence from California (GenBank accession no. DQ059764). NCSU strain 06-COI is available upon request for research purposes.

We report the isolation of *B. vinsonii* subsp. *berkhoffii* genotype III from a military working dog that originated in Germany and was stationed in Texas for 18 months prior to the diagnosis of bartonella endocarditis. When the ITS sequence was compared to an ITS sequence from a California gray fox (GenBank accession no. DQ059764) (14), the percent identity was lower (98.9%) than comparable identities (99.9 to 99.7%) with sequences from Europe or China. As persistent intravascular infection for 14 months with a *B. vinsonii* subsp. *berkhoffii* genotype II strain in a healthy dog from North Carolina was reported (11), it is possible, based upon ITS sequence similarities, that the dog was infected in Germany prior to shipment to the United States. Alternatively, the dog may have been coinfected with *E. canis* and *Bartonella vinsonii* subsp. *berkhoffii*. Previous studies have reported a seroepidemiological association between exposure to *Bartonella vinsonii* subsp. *berkhoffii* and to *E. canis*, suggesting potential cotransmission by *Rhipicephalus sanguineus* (16). Whether ITS sequence differences among dog and fox strains reflect host adaptation, strain variation due to geographic origin, or random events remains unanswered. Among the *Bartonella* species, *B. vinsonii* subsp. *berkhoffii* appears to have evolved to preferentially infect canines, including coyotes, dogs, and gray foxes. Those factors that result in disease expression in dogs, including but not limited to endocarditis, are most likely multifactorial and are not well understood. Obtaining additional *B. vinsonii* subsp. *berkhoffii* genotype data should better define the reservoir potential, carrier patterns, modes of transmission, and geographic distribution of these zoonotic organisms in nature.

Nucleotide sequence accession numbers. GenBank accession numbers include EU295657, 16S rRNA gene and 16S-23S intergenic spacer region partial sequence; EU295660, *pap31* partial sequence; EU295661, *rpoB* partial sequence; EU295658, *gdh1* partial sequence; and EU295659, *ialB* partial sequence.

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