

Persistent Infection of Pets within a Household with Three *Bartonella* Species

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We monitored by blood culture and immunofluorescence assay (IFA) bartonella infection in one dog and eight cats in a household to determine the prevalence and persistence of the infection as well as its transmissibility to humans. Ectoparasite control was rigorously exercised. During a 3-year period, *Bartonella clarridgeiae* was recovered from one cat on two occasions, and *B. henselae* was isolated from another cat on four occasions. During a 16-month period, *B. vinsonii* subsp. *berkhoffii* was isolated from the dog on 8 of 10 culture attempts. Despite extensive household contact, the pet owner was seronegative to all three species by IFA for *Bartonella*-specific immunoglobulin G.

Bartonella species are hematotropic bacteria that have been isolated from humans, cats, dogs, wildlife species, rodents, and arthropods. The mammalian reservoir for *B. clarridgeiae* and *B. henselae* is the domestic cat, in which asymptomatic infection can be maintained for long periods (1–3). Feline infection with *B. henselae* is widespread throughout the world, and *B. clarridgeiae* has been isolated from cats in Europe and various regions of the United States (4–6). Chomel et al. have demonstrated flea transmission of *B. henselae* between cats (7). Prolonged bacteremia has been demonstrated in cats, but to our knowledge persistent asymptomatic *B. vinsonii* subsp. *berkhoffii* infection in dogs, as described in this report, has not been previously documented. Human exposure to *B. henselae* can result in cat-scratch disease (CSD) or other medical conditions (8), and *B. clarridgeiae* was recently implicated as the cause of CSD in a veterinarian (6).

B. vinsonii was originally isolated by Baker in 1946; splenic homogenates were cultured from voles that inhabited a former immigrant quarantine station on Grosse Isle, Canada (9). No other isolates of *B. vinsonii* were described until 1995 when we recovered a similar organism from a dog with valvular endocarditis (10). Subsequent

genotypic evaluation of the vole and canine isolates resulted in taxonomic division of the species into subspecies. The vole and canine isolates were designated *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *berkhoffii*, respectively (11). Although the type strain of *B. vinsonii* subsp. *berkhoffii* was isolated from a dog with cardiac disease, the organism can also cause subclinical infection. The reservoir for *B. vinsonii* subsp. *berkhoffii* has not been established, but seroepidemiologic evidence supports the potential for tick transmission (12).

We describe infection of three pets within the same household with three different *Bartonella* species and the subclinical persistence of *B. vinsonii* subsp. *berkhoffii* infection in a dog for 14 months. This report is a component of other ongoing studies of *Bartonella* infection in animals. In conjunction with an earlier study, a veterinary student offered her cats as controls for an investigation into the prevalence of *B. henselae* associated with cases of CSD (1). Three cats and the dog were subsequently monitored for bacteremia and seroreactivity during a bartonella antimicrobial efficacy study, in which the cats received 22.7 mg enrofloxacin q12h PO for 28 days and the dog received 25 mg doxycycline q12h PO for 28 days (13).

Blood samples were collected from the dog and additional cats acquired later by the student. At each collection, all animals were assessed for general health. For the dog, packed cell volume and total plasma protein were determined from EDTA blood samples.

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Dispatches

Sera were analyzed by immunofluorescence antibody assay (IFA) for reactivity against *B. clarridgeiae*, *B. henselae*, and *B. vinsonii* subsp. *berkhoffii* (1). All positive serum samples (dog, cat, and human) were reassayed at least three separate times.

Blood samples from the dog and cats were placed into 1.5-ml pediatric isolator tubes (Wampole Laboratories, Cranbury, NJ) for *Bartonella* culture on rabbit blood agar (1). Isolates were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the 16S rRNA gene and 16S-23S intergenic spacer (ITS) region using *Dde* I, *Mnl* I, *Hae* III, and *Alu* I restriction endonuclease (6). DNA from the type strains of *B. clarridgeiae* (ATCC 51734), *B. henselae* (ATCC 49882), and *B. vinsonii* subsp. *berkhoffii* (ATCC 51672), in addition to *B. vinsonii* subsp. *berkhoffii* strains cultured from two healthy dogs, was analyzed for comparison.

The dog, cats, and pet owner remained healthy throughout the study. The packed cell volume and total plasma protein values of the dog remained within reference ranges. These values were not routinely assessed in the cats. The source, age, and order of introduction of animals into the household are depicted in Table 1. *Bartonella*-specific immunoglobulin G (IgG) was detected at various titers in the animals (Table 2).

During the 3-year period (December 1993 to January 1997), *B. clarridgeiae* was recovered from one cat on two occasions, and *B. henselae* was isolated from another cat on four occasions. *B. vinsonii* subsp. *berkhoffii* was isolated from

Table 1. Animals included in the study

Animal	Breed ^a	Sex ^b	Birth-date	Date acquired	Source/state
Cats					
A	DSH	M/C	10/91	12/91	Pet store/CA
B	DSH	F/S	10/90	12/90	Stray/DE
C	DLH	F/S	6/92	6/92	Stray/NC
D	DSH	M/C	4/93	5/93	Stray/NC
E	DSH	F/S	5/93	7/93	Stray/NC
F	DLH	M/C	4/94	6/94	Stray/NC
G	DLH	F/S	6/95	10/95	Stray/NC
H	MC	M/C	6/96	9/96	Family/NC
Dog					
I	PM	F/S	4/95	6/95	Family/NC

^aDSH, Domestic shorthair; DLH, Domestic longhair; MC, Maine coon; PM, Pekingese mix.

^bM/C, Male/castrated; F/S, Female/spayed.

Table 2. Blood culture and immunofluorescence antibody assay results

Date	ID	Culture results ^a	Colony counts/ml	Serologic results		
				Bh	Bc	Bvb
12/4/93	A	+ (Bh)	97	256	32	512
	D	-		ND ^b	ND	ND
1/15/94	C	+ (Bc)	67	<16	<16	<16
5/25/94	A	+ (Bh)	13	128	16	256
	C	+ (Bc)	113	128	<16	<16
	D	-		256	1024	512
8/19/94	A	cont		128	<16	128
	C	+ (Bc)	240	64	<16	<16
9/18/94	F	nd		<16	<16	<16
12/9/94 ^c	A	-		32	<16	128
12/13/94	C	-		32	<16	64
	D	-		128	1024	512
5/6/95 ^d	A	-		64	<16	64
	C	+ (Bc)	9	32	<16	32
	F	-		<16	<16	<16
5/27/95	A	-		64	16	64
	C	-		<16	<16	<16
	F	-		<16	<16	<16
6/10/95	A	-		32	<16	64
	C	-		32	<16	<16
	F	-		<16	<16	<16
6/22/95	A	-		32	<16	128
	C	-		<16	<16	32
	F	-		<16	<16	<16
7/30/95	A	-		32	<16	256
	C	-		32	<16	<16
	F	-		<16	<16	<16
11/19/95	A	-		16	<16	256
	B	-		<16	<16	32
	C	-		32	<16	32
	D	-		64	<16	64
	E	-		<16	<16	256
	F	-		<16	<16	32
	G	-		32	<16	128
	I	+ (Bvb)	>1000	128	<16	512
	I	+ (Bvb)	369	64	<16	256
2/25/96	I	+ (Bvb)	164	32	<16	256
3/24/96	I	+ (Bvb)	154	16	<16	128
4/21/96	I	+ (Bvb)	357	16	<16	128
6/22/96	I	+ (Bvb)	12	<16	<16	64
8/24/96	I	+ (Bvb)	12	<16	<16	64
10/21/96	I	-		<16	<16	64
12/18/96	I	+ (Bvb)	6	16	<16	64
1/22/97	student		nd	32	16	32
1/14/97	A	-		16	<16	128
	B	-		<16	<16	<16
	C	-		<16	<16	16
	D	-		64	<16	64
	G	-		<16	<16	32
1/30/97 ^e	I	+ (Bvb)	1	16	<16	64
	I	-		16	<16	32

^aBh, *B. henselae*; Bc, *B. clarridgeiae*; Bvb, *B. vinsonii* subsp. *berkhoffii*; cont, contaminated; nd, not done.

^bND, no data.

^cFour weeks before sample was drawn, the cat received amoxicillin clavulanate (62.5 mg q12h, for 10 days) for the treatment of an oral abscess.

^dAfter collection of blood samples, these animals received enrofloxacin at 22.7 mg q12h for 28 days.

^eAfter collection of blood samples, the dog received 28 days of doxycycline hyclate at 25 mg q12h for 28 days.

the dog on 8 of 10 culture attempts during 16 months of observation (Table 2). Only one species of *Bartonella* was identified from any individual animal. In all three animals, blood cultures performed after completion of antimicrobial therapy were negative.

When compared with type strains, PCR-RFLP analysis of the 16S rRNA gene and the 16S-23S ITS region identified the feline isolates as *B. clarridgeiae* or *B. henselae*. Endonuclease digestion of the 16S gene of the isolates from the dog as well as two isolates from other subclinically bacteremic dogs produced restriction patterns identical to the type strain of *B. vinsonii* subsp. *berkhoffii*; however, digestion of the ITS region resulted in a different restriction profile for the subclinically bacteremic dogs (Figure).

We did not detect bacteremia temporally associated with exposure to known bacteremic animals in any pet newly introduced to the household. Chomel et al. (7) reported that as few as five infected fleas could transmit *B. henselae* infection between cats, but normal daily contact between infected and uninfected cats in ectoparasite-free surroundings was insufficient to produce bacteremia in the uninfected cats. Despite their immunologic status (seronegative) and the occasional presence of fleas, some cats in this household did not seroconvert or become bacteremic after continued exposure to infected resident animals. Although we have observed that infection with *B. henselae* is not cross-protective against *B. clarridgeiae* (Kordick and Breitschwerdt, unpub. data) and that dual infection with *B. clarridgeiae* and *B. henselae* can occur in cats (2,6), similar data are not available with regard to *B. vinsonii* subsp. *berkhoffii*. *B. vinsonii* subsp. *berkhoffii* bacteremia has not been reported in cats. Intradermal injection of *B. henselae* into dogs has induced seroconversion but not bacteremia (14). It is therefore not surprising that *B. vinsonii* subsp. *berkhoffii* was not recovered from any of the cats in the study or, conversely, that *B. henselae* or *B. clarridgeiae* were not recovered from the dog.

Interpretation of *Bartonella* IFA serologic results for species identification has been compromised because of presumed cross-reactivity. The dog in this study never seroconverted to *B. clarridgeiae* or *B. henselae*; however, the cat infected with *B. henselae* had a consistently elevated level of antibodies reactive against *B. vinsonii* subsp. *berkhoffii* antigen. The issue

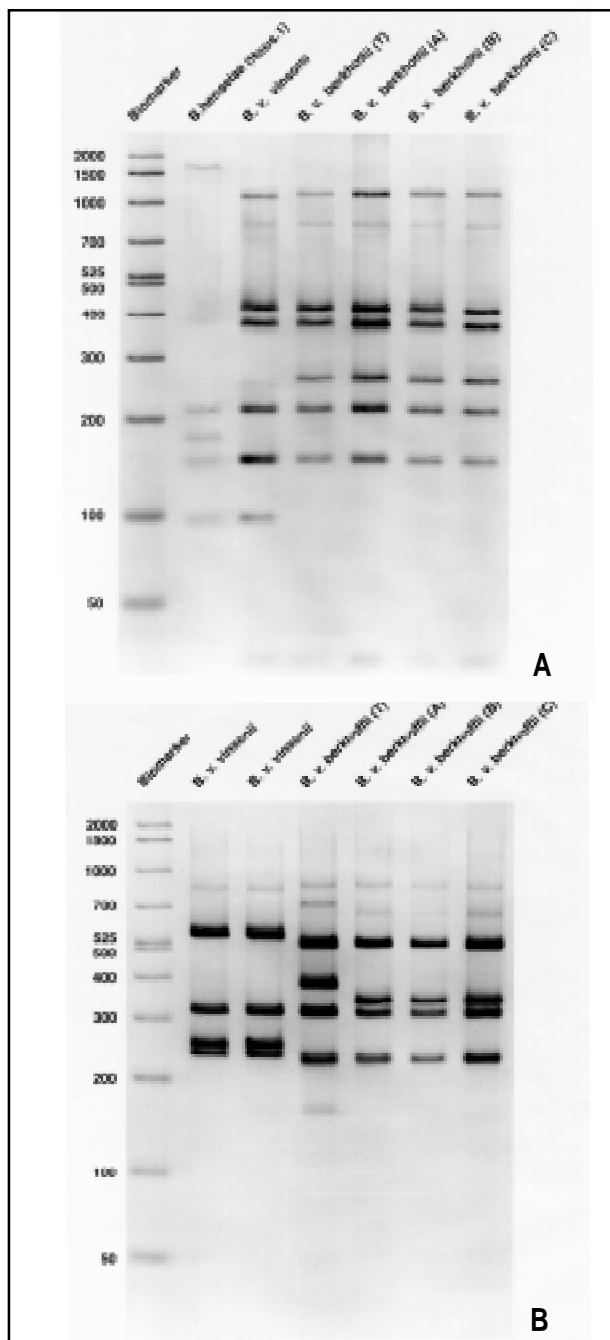


Figure. A. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the 16S rRNA gene of *B. vinsonii* subsp. *berkhoffii* isolates using *Dde* I demonstrating differentiation between subspecies of *B. vinsonii*. B. PCR-RFLP analysis of the 16S-23S intergenic spacer region of *B. vinsonii* subsp. *berkhoffii* isolates using *Hae* III. Strain differences were detected between *B. vinsonii* subsp. *berkhoffii* (T), which was the type strain cultured from a dog with endocarditis, and isolates A (described in this report), B, and C from subclinically infected dogs.

of serologic cross-reactivity among *Bartonella* species deserves additional study.

Subtyping of bacteria after endonuclease digestion of the 16S-23S rRNA ITS region has been performed by other investigators because the spacer region, not appearing to encode functional proteins, demonstrates sequence variability in an operon that has been useful for taxonomic classification (15,16). In this study, different restriction profiles were obtained from isolates derived from asymptomatic dogs compared with the type strain obtained from a dog with endocarditis. Since our sample size was small, further examination of these isolates by pulsed-field gel electrophoresis or bacteriophage analysis may determine whether PCR-RFLP analysis is epidemiologically useful in predicting the pathogenicity of different strains.

The pet owner (student) remained healthy throughout the study; however, she reported that 2½ years before the study she had debilitating fatigue of 1 month duration without fever or lymphadenopathy. At the time, she lived in California and had recently acquired Cat A. A mononucleosis test was negative; her physician suspected a viral infection. Whether the fatigue was related to *Bartonella* exposure (CSD) or the cat was bacteremic is not possible to determine retrospectively; however, the cat was bacteremic with *B. henselae* when cultured 2½ years later as part of another study (1). *Bartonella* transmission from dogs and cats to humans appears to be relatively inefficient. It is not surprising, therefore, that despite continuous exposure to three *Bartonella* species, the student's serum sample was minimally reactive to *Bartonella* antigens.

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