

## *Bartonella* Species Bacteremia in Two Patients with Epithelioid Hemangioendothelioma<sup>∇</sup>

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***Bartonella henselae* and *B. koehlerae* bacteremia was documented in two epithelioid hemangioendothelioma patients and *B. koehlerae* bacteremia in an asymptomatic partner of one of the patients. Considering the biology and clinically variable natural history of epithelioid hemangioendothelioma, these results suggest that chronic *Bartonella* infection could have a role in the development of this vascular neoplasm. *Bartonella* spp. are known to induce vasoproliferative tumors in immunocompromised patients and may play a role in the development of epithelioid hemangioendothelioma in immunocompetent patients.**

### CASE REPORTS

Case 1 was a 37-year-old female who was born in Poland and had lived in Australia for the previous 30 years. Prior to the present illness, she had been generally healthy and routinely participated in a variety of outdoor recreational activities, including advanced skiing, soccer, and competitive cycling. She traveled extensively throughout the Americas, Europe, and Southeast Asia. The patient had experienced numerous insect bites, thought to be fleas, in a San Francisco backpackers' hostel in 1997, which was followed by a brief hospitalization due to cellulitis. Between 1976 and 2011, she had four pet dogs and two pet cats but did not recall seeing fleas on her pets.

Beginning in January 2008, the patient experienced sharp pain in the right upper quadrant of her abdomen; biliary colic was suspected, and the pain resolved within 7 days. Subsequently, abdominal ultrasound and computed tomography (CT) revealed nine distinct lesions, the largest measuring 5.4 cm (segments 5 and 6), 3.1 cm (segment 4b), 2.5 cm (segment 4a), and 2.3 cm (segment 2) in diameter. In February 2008, laparoscopic core and wedge biopsies resulted in a diagnosis of hepatic epithelioid hemangioendothelioma (EHE). The patient underwent a comprehensive liver transplant workup. Lung CT scans performed in 2008 revealed several subcentimeter lung nodules, which were unchanged through 2009. Liver transplantation was advised as the best course of treatment to prevent metastatic disease. The patient elected serial

CT scans and was removed from the active liver transplant list. Hashimoto's thyroiditis was diagnosed in December 2008 and treated with iodine supplementation.

Subsequently, three sequential abdominal CT scans identified lesional growth during the next 12 months, and by June of 2009, abdominal CT scans identified 13 lesions and indicated that the two largest lesions were starting to coalesce. The patient elected treatment by multiple irreversible electroporation (IRE). Following the diagnosis of EHE, the patient continued to play soccer and cycle, and her test results remained in the normal range except after each IRE treatment (29). Blood results remained normal, with the exception of indications of elevated liver enzyme activity (as determined by alkaline phosphatase [ALP], alanine aminotransferase [ALT], aspartate aminotransferase [AST], and gamma-glutamyl transferase [GGT] levels) following IRE.

Because of prior detection of *Bartonella vinsonii* subsp. *berkhoffii* in an EHE patient (8), a liver biopsy specimen (case 1 only) and blood and serum samples from this EHE patient and an EHE patient from England (case 2) were submitted by the attending physicians to the North Carolina State University Intracellular Pathogens Research Laboratory (IPRL) for attempted isolation or molecular detection of a *Bartonella* species. A DNeasy tissue kit protocol (Qiagen Inc., Valencia, CA) was used to extract DNA from tissue scrolls cut from the paraffin-embedded liver tissues containing the EHE tumor from patient 1, followed by PCR targeting the 16S-23S intergenic spacer (ITS) region (3, 7, 31).

Because both patients resided in distant countries, samples were submitted in batches consisting of three blood and serum sample sets obtained at intervals of one to two days. All patient samples were tested as a component of an Institutional Review Board (IRB)-approved study entitled *Detection of Bartonella Species in the Blood of People with Extensive Animal Contact* (North Carolina State University Institutional Review Board; IRB 64-08-05).

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We used a previously described microbiological approach incorporating enrichment cultures of patient blood and serum in *Bartonella* alphaproteobacteria growth medium (BAPGM), followed by PCR targeting the 16S-23S ITS region (3, 7, 14, 16). The BAPGM diagnostic platform consists of PCR analysis performed following direct extraction of DNA from blood and serum, enrichment cultures from days 7 and 14, and PCR when colony growth is seen following subculture growth on blood agar plates. Bacterial isolation, PCR amplification, and cloning were performed using previously described methods (3, 7, 14, 16). Sequences were aligned and compared with GenBank sequences by the use of AlignX7 software (Vector NTI Suite 6.0; InforMax, Inc.) (14). To assess potential laboratory contamination, an uninoculated BAPGM culture flask was processed simultaneously and in the same manner with each batch of patient blood and serum samples tested. Specifically, while establishing cultures from a batch of samples in a biosafety hood, the top was removed from the BAPGM uninoculated control flask until all patient samples had been processed. Following the standard operating procedures in the IPRL, sample preparation, including BAPGM culture and agar plate subinoculation, DNA extraction, PCR preparation, and PCR amplification and analysis, was performed in separate laboratory rooms to avoid culture as well as DNA contamination. In addition, negative and positive *Bartonella* PCR controls consisted of DNA extracted from blood free of bacteria and the same DNA spiked with *B. henselae* genomic DNA at 0.5 genome copies/ $\mu$ l, respectively. PCR controls were used for each batch of DNA tested, and negative controls remained negative throughout the study period.

*Bartonella koehlerae*, *B. vinsonii* subsp. *berkhoffii* (genotypes I, II, and III), and *B. henselae* antibody levels were determined following traditional immunofluorescence antibody assay (IFA) practices (5, 8) with fluorescein-conjugated goat anti-human IgG (Pierce Biotechnology, Rockford, IL). *Bartonella vinsonii* subsp. *berkhoffii* genotype I (NCSU CO-1-93) was isolated from a dog with endocarditis. *B. koehlerae* (NCSU FO-1-09) and *B. henselae* strain Houston-1 (NCSU FO-93-23) were isolated from naturally infected cats. *Bartonella* organisms were passed from agar-grown cultures into DH82 cell cultures to obtain IFA antigens. Heavily infected cell cultures were spotted onto 30-well Teflon-coated slides (Cel-Line; Thermo Scientific), dried in air, fixed in acetone, and stored frozen. Serum samples were diluted in phosphate-buffered saline (PBS) solution containing normal goat serum, Tween 20, and powdered nonfat dry milk to block nonspecific antigen binding sites. All available patient sera were screened at dilutions of 1:16 to 1:64. All sera that were reactive at 1:64 were further tested with twofold dilutions out to 1:8, 192. A cutoff titer of 1:64 was considered to represent seroreactivity.

Initially, *B. koehlerae* was amplified and sequenced from a stored paraffin-embedded hepatic EHE tissue sample from case 1 obtained in Australia in February 2008. In July 2009, blood and serum were submitted for *Bartonella* serology, enrichment blood culture, and serial PCR testing as a component of the BAPGM platform (Table 1). Testing of the first group of serum and blood samples (July 2009) yielded antibodies to all five test antigens. One of three blood samples was PCR positive, but efforts to sequence the amplicon were unsuccessful. Subsequently, *B. vinsonii* subsp. *berkhoffii* genotype I, *B. hense-*

*lae* (strain SA2), and *B. koehlerae* were detected on one, three, and six occasions, respectively. At two time points, PCR results were positive, but efforts to sequence the amplicons were not successful. For 12 months, beginning in September 2009, the patient received azithromycin (500 mg daily) and rifampin (600 mg once daily). During this period, seroreactivity persisted, with *Bartonella* antibody titers ranging from 1:64 to 1:1,024, and *Bartonella* DNA sequences were detected in blood or BAPGM enrichment culture samples on several occasions (Table 1). Reviews of liver CT scans spanning a 15-month time period up to September 2010 showed no evidence of tumor progression, and a repeat PET scan (UDP glucose) revealed reduced tracer avidity.

Due to concerns related to horizontal transmission among partners, oral swabs from both individuals were tested. *B. koehlerae* DNA was amplified and sequenced from one of three swabs obtained from the patient in September 2010. Serology was performed, and six oral swabs and three blood cultures from the patient's cat were concurrently tested. The cat's antibody titers were <1:16, and no DNA was amplified from the oral swabs, the blood, the serum, or the BAPGM enrichment cultures from days 7 and 14.

Beginning in September 2010, no antibiotics were administered for 6 months, during which time the patient remained asymptomatic. However, when *B. henselae* DNA was again amplified and sequenced from blood and from a BAPGM enrichment culture in October, doxycycline and rifampin treatment was restarted in April 2011. Retesting in March and May 2011 identified a decrease in seroreactivity to undetectable levels for *B. koehlerae* and *B. vinsonii* subsp. *berkhoffii* genotype I and II antigens. *Bartonella* DNA was not amplified from the March or May blood, serum, or enrichment blood cultures, and subcultures did not result in bacterial growth.

Case 2 was a 51-year-old female born in England. Retinoblastoma was diagnosed at 30 months of age. As an adult, this individual held an administrative position with minimal historical animal contact. She had traveled to Africa, Asia, Australia, Europe, and North America. She reported insect bites by Blandford flies in the United Kingdom that required antibiotic administration due to delayed healing. In December 2005, the patient presented for upper right quadrant abdominal pain. Ultrasound and a subsequent CT scan showed multiple hepatic lesions. A liver biopsy specimen obtained in January 2006 contained hepatic tissue with atypical infiltrate of vacuolated cells, some of which lined vascular channels. There was no evidence of metastatic carcinoma or lymphoma. The histopathological diagnosis was EHE. A 7-month course of gamma interferon treatment resulted in minimal improvement. When a laparoscopy was performed in June 2007, there were signs of mild peritoneal invasion. Peritoneal biopsy specimens included nodular fibrous tissue containing plump vacuolated epithelial cells that were CD34 and CD31 positive. The cells were similar to those observed in the 2006 liver biopsy specimen. Additional cancer treatment was not recommended. By March 2008, the patient reported an increased frequency of abdominal pain in the right upper quadrant. A magnetic resonance imaging (MRI) scan identified growth of some tumors, whereas other hepatic lesions had decreased in size, changes attributed to the gamma interferon treatment. From June 2008 to June 2010, the patient experienced occasional fatigue, headaches, irrita-

TABLE 1. Serological and PCR results for patient 1 with EHE

Sample date (mo/day/yr)	IFA reciprocal titer					ITS PCR result <sup>a</sup>		
	<i>Bartonella vinsonii</i> subsp. <i>berkhoffii</i> genotype			<i>B. henselae</i>	<i>B. koehlerae</i>	Blood	BAPGM enrichment blood culture	Other sample
	I	II	III					
4/17/2009								<i>Bk</i> (tissue)
7/15/2009	128	256	256	128	>256	Neg	Neg	
7/17/2009	128	128	256	64	>256	<i>Bspp</i>	Neg	
7/20/2009	256	128	128	64	>256	Neg	Neg	
11/23/2009	256	1,024	128	256	256	<i>BhSA2</i>	<i>BhSA2</i>	
11/26/2009	1,024	1,024	512	256	<16	<i>BvbI</i>	Neg	
11/30/2009	512	1,024	256	512	<16	Neg	Neg	
1/20/2010	1,024	256	128	256	32	<i>Bk</i>	Neg	
1/22/2010	512	256	128	256	64	Neg	Neg	
1/25/2010	1,024	128	128	64	32	Neg	Neg	
3/5/2010	1,024	512	256	64	1,024	<i>Bk</i>	Neg	
3/8/2010	256	128	256	64	512	Neg	<i>Bk</i>	
3/10/2010	<16	<16	256	128	<16	<i>Bk</i>	Neg	
6/2/2010	512	1,024	128	512	128	Neg	Neg	
6/4/2010	1,024	512	128	512	128	Neg	<i>BhSA2</i>	
6/7/2010	512	512	128	512	256	Neg	Neg	
9/8/2010	512	128	256	256	256	Neg	Neg	<i>Bk</i> (OS)
9/10/2010	512	256	256	256	512	Neg	Neg	Neg (OS)
9/13/2010	512	256	256	256	256	Neg	Neg	Neg (OS)
10/13/2010	>256	256	128	256	64	Neg	<i>Bh</i>	
10/15/2010	64	256	256	256	64	<i>BhSA2</i>	<i>BhSA2</i>	
10/18/2010	64	256	128	256	64	Neg	Neg	
12/8/2010	64	512	64	128	64	Neg	Neg	
12/10/2010	64	512	128	256	64	Neg	Neg	
12/13/2010	64	256	64	128	64	<i>Bspp</i>	Neg	
3/24/2011	256	128	128	256	<16	Neg	Neg	
3/25/2011	128	64	128	64	<16	Neg	Neg	
3/28/2011	64	32	64	64	<16	Neg	Neg	
5/18/2011	<16	<16	256	64	<16	Neg	Neg	
5/20/2011	<16	<16	<16	<16	<16	Neg	Neg	
5/23/2011	<16	<16	<16	<16	<16	Neg	Neg	

<sup>a</sup> Neg, negative; OS, oral swab; *Bspp*, *Bartonella* genus PCR positive (unable to sequence amplicon); *BhSA2*, *Bartonella henselae* strain San Antonio 2; *BvbI*, *Bartonella vinsonii* subsp. *berkhoffii* genotype I; *Bk*, *Bartonella koehlerae*.

bility, blurred vision, eye pain, insomnia, loss of sensation in fingers and toes, and joint pain. There was minimal change in lesion size between the June 2008 CT scan and a scan obtained in June 2010. No anticancer treatments were administered during that time.

When a single blood and serum sample set was tested in the IPRL in April 2010, the patient was not seroreactive to any of the five test antigens. However, *B. koehlerae* DNA was ampli-

fied and sequenced from a BAPGM enrichment blood culture (Table 2). When three sample sets were retested in August 2010, the patient was again nonseroreactive, and, with the exception of a positive PCR result following DNA extraction from a blood sample, all other components of the BAPGM platform were PCR negative. Attempts to sequence the amplicon were not successful. When three sample sets were retested in November 2010, antibody reactivity to all five test

TABLE 2. Serological and PCR results for patient 2 with EHE

Sample date (mo/day/yr)	IFA reciprocal titer					ITS PCR result <sup>a</sup>		
	<i>Bartonella vinsonii</i> subsp. <i>berkhoffii</i> genotype			<i>B. henselae</i>	<i>B. koehlerae</i>	Blood	BAPGM enrichment blood culture	Other sample
	I	II	III					
1/6/2011								Neg (tissue)
6/1/2007								Neg (tissue)
8/4/2010	<16	<16	<16	<16	<16	Neg	Neg	
8/5/2010	<16	<16	<16	<16	<16	Neg	Neg	
8/6/2010	<16	<16	<16	<16	<16	<i>Bspp</i>	Neg	
11/1/2010	>256	64	64	<16	64	Neg	Neg	
11/3/2010	>256	64	128	64	64	Neg	Neg	
11/5/2010	>256	128	128	<16	64	<i>BhSA2</i>	Neg	

<sup>a</sup> Neg, negative; *BhSA2*, *Bartonella henselae* strain San Antonio 2; *Bspp*, *Bartonella* genus PCR positive (unable to sequence amplicon to confirm species and strain).

TABLE 3. Serological and PCR results for the boyfriend of patient 1 and the husband of patient 2

Subject <sup>a</sup>	Sample date (mo/day/yr)	IFA reciprocal titer					ITS PCR result <sup>b</sup>		
		<i>Bartonella vinsonii</i> subsp. <i>berkhoffii</i> genotype			<i>B. henselae</i>	<i>B. koehlerae</i>	Blood	BAPGM Enrichment Blood Culture	Other sample
		I	II	III					
B	6/2/2010	<16	<16	<16	<16	32	Neg	<i>Bk</i>	
B	6/4/2010	<16	<16	<16	<16	<16	Neg	Neg	
B	6/7/2010	<16	<16	<16	<16	<16	Neg	<i>Bk</i>	
B	9/8/2010	<16	<16	<16	<16	<16	<i>Bk</i>	Neg	Neg (OS)
B	9/10/2010	<16	<16	<16	<16	<16	Neg	Neg	Neg (OS)
B	9/13/2010	<16	<16	<16	<16	<16	Neg	Neg	Neg (OS)
B	10/13/2010	64	<16	128	16	<16	<i>Bspp</i>	Neg	
B	10/15/2010	<16	<16	<16	<16	<16	Neg	Neg	
B	10/18/2010	>256	<16	64	64	<16	Neg	Neg	
B	12/8/2010	<16	<16	<16	<16	<16	Neg	Neg	
B	12/10/2010	<16	<16	<16	<16	<16	Neg	Neg	
B	12/13/2010	<16	<16	<16	<16	<16	Neg	Neg	
H	11/1/2010	64	64	64	<16	<16	Neg	Neg	
H	11/3/2010	64	64	64	<16	<16	Neg	Neg	
H	11/5/2010	64	16	64	32	<16	Neg	Neg	

<sup>a</sup> B, boyfriend of patient 1, H, husband of patient 2.

<sup>b</sup> Neg, negative; OS, oral swab; *Bk*, *Bartonella koehlerae*; *Bspp*, *Bartonella* genus (PCR positive, unable to sequence amplicon).

antigens was found and *B. henselae* (strain SA2) DNA was amplified and sequenced from one blood sample. In the November sample batch, antibody titers were generally within one dilution of each other; however, as with patient 1, there was a 4-fold or greater (<16, 64, <16) variation in *B. henselae* reciprocal titers corresponding to the three sample collection dates.

**Partner of patient 1.** This 40-year-old male office worker resided in Australia. He had frequent exposure to cats and occasional exposure to dogs but reported infrequent exposure to insects. He had traveled to North and Central America. He was healthy, had not previously sought medical care, and reported no historical or current symptoms. In June and September of 2010, he was seronegative for all five test antigens; however, *B. koehlerae* DNA was amplified and sequenced from two BAPGM enrichment blood cultures obtained in June and from a September blood sample (Table 3). *B. koehlerae* DNA was not amplified from three oral swabs that had been obtained in September, prior to antibiotic treatment. Despite being healthy, and because of repeated documentation of *B. koehlerae* infection in his girlfriend (case 1), the boyfriend was treated for *B. koehlerae* bacteremia with azithromycin (500 mg daily) for 14 days beginning 7 September 2010. Following antibiotic treatment for *B. koehlerae* bacteremia, he was variably seroreactive (titers of 1:64 or 1:128) to three test antigens in October, but seroreactivity to either *B. koehlerae* or *B. vinsonii* subsp. *berkhoffii* genotype II antigens was not detected (Table 3). Despite documentation of *B. koehlerae* infection, the patient never seroconverted to that *Bartonella* antigen. PCR results from tests performed with blood, serum, and enrichment blood cultures obtained in November and December 2010 were negative, and antibodies were no longer detectable in the three December serum samples.

The husband of patient 2 was a 58-year-old company director who reported infrequent animal contact and occasional exposure to mosquitoes and biting flies (Blandford flies). This person had traveled to Africa, Asia, Australia, Europe, and the United States. He reported infrequent illness accompanied by

irritability, muscle pain, loss of sensation, and joint pain. During the previous 5 years, he had experienced chronic neck and back pain and was diagnosed with non-insulin-dependent diabetes mellitus. He was seroreactive to *Bartonella vinsonii* subsp. *berkhoffii* antigens (all at a titer of 1:64); however, *Bartonella* DNA was not amplified from blood or BAPGM enrichment blood cultures (Table 3).

Previously, we reported infection with *Bartonella vinsonii* subsp. *berkhoffii* genotype II in a patient with EHE (8). In this report, we describe sequential documentation of *B. henselae*, *B. koehlerae*, and *B. vinsonii* subsp. *berkhoffii* genotype I bacteremia in one EHE patient, concurrent *B. koehlerae* bacteremia in the asymptomatic partner of patient 1, and *B. henselae* and *B. koehlerae* bacteremia in a second EHE patient. Epithelioid hemangioendothelioma is a vascular neoplasm of endothelial origin, with malignant potential ranging between benign hemangioma and angiosarcoma. First described by Weiss and Enzinger in 1982 (30), EHE is characterized by positive immunostaining results for endothelial antigens CD34 and factor VIII. This uncommon cancer usually localizes to hepatic, pulmonary, cutaneous, or bony locations, with the resultant symptoms defined by the site of tumor localization. The natural history of EHE is extremely unpredictable, and there is substantial interpatient variability in disease progression. Intervals of rapid growth are frequently interrupted by long periods of quiescence; however, EHE is generally unresponsive to standard cytotoxic chemotherapy and radiation therapy. Considering the unusual biology and clinically variable natural history of EHE, it seems possible that chronic *Bartonella* infection could have a causal relationship with the development of this cancer. The unique capability of *Bartonella* to invade and induce long-lasting intraerythrocytic and intraendothelial infections, in conjunction with the ability of at least three *Bartonella* spp. (*B. henselae*, *B. quintana*, and *B. bacilliformis*) to induce vascular



endothelial growth factor (VEGF)-mediated vasoproliferative disease in immunocompromised or immunocompetent individuals, suggests that these novel emerging bacterial pathogens might contribute to the development of vascular tumors in immunocompetent as well as immunocompromised patients (11, 12).

*Bartonella* is the only bacterial genus known to cause endothelial proliferation, presumably by inducing aberrant angiogenic signaling in a manner that is analogous to the angiogenic pathogenesis in malignant tumors (13). In addition, *Bartonella*-induced VEGF contributes to noncancerous angioproliferative diseases such as bacillary angiomatosis and peliosis hepatis, most often in immunocompromised patients (10, 12, 20, 27). On a comparative medical basis, we have previously reported blood culture isolation of *B. vinsonii* subsp. *berkhoffii* genotype II from a dog with a hemangiopericytoma and from a dog with bacillary angiomatosis that was concurrently receiving immunosuppressive drug therapy (8, 31). The association of increased tissue VEGF levels with hemangioendotheliomas also lends credence to the hypothesis that *Bartonella* infection may be a cause or cofactor in the development of hemangioendotheliomas in some patients (22, 30). *Bartonella henselae* causes vasoproliferative lesions in patients with bacillary angiomatosis and bacillary peliosis (26), and in the context of disease causation, these lesions regress after successful antibiotic treatment (18). On the basis of *in vitro* infection of human endothelial cell lines, *B. henselae* has been shown to induce angiogenesis and endothelial cell proliferation (24). *Bartonella* spp. upregulate mitogenic and proinflammatory genes, resulting in cytoskeletal rearrangement and suppression of endothelial cell apoptosis (11, 12). Infection of human endothelial cells by *B. henselae* resulted in interleukin-8 (IL-8) production and upregulation of IL-8 receptors such as CXCR2 (19, 25). IL-8 promotes angiogenesis through enhanced endothelial cell survival and enhanced vascular proliferation (19, 20, 23, 25). Whether *B. koehlerae* can induce angiogenesis through the production of hypoxia-inducible factor and vascular endothelial growth factor is unknown. The findings regarding the two EHE patients described in this study, in conjunction with those determined for a previously described EHE patient infected with *B. vinsonii* subsp. *berkhoffii* genotype II, suggest that the association between *Bartonella* infection and EHE should be systematically investigated.

Based upon the serological and sequential PCR test results determined for case 1, it seems likely that the woman was coinfecting with three different *Bartonella* species when the first batch of blood samples was submitted for *Bartonella* testing. Because different *Bartonella* species were amplified and sequenced from blood samples from both patients at different time points, it is not possible to confirm whether these patients were coinfecting or sequentially infected during the course of study. As *B. koehlerae* was amplified and sequenced from the EHE liver biopsy sample obtained in February 2008 and from blood and BAPGM enrichment blood cultures tested in March 2010, persistent intravascular infection seems likely. Similarly, *B. henselae* strain SA2 was amplified and sequenced from blood samples obtained in November 2009 and in June and October 2010. Fewer sample sets were available for sequential testing from patient 2, and only *B. koehlerae* was detected after enrichment culture. Recently, *B. koehlerae* bacteremia was

documented in eight immunocompetent patients with a spectrum of chronic arthritic, cardiovascular, and neurological symptoms and in a girl with peripheral visual deficits, a sensory neuropathy, and hallucinations (5, 6, 9). *Bartonella vinsonii* subsp. *berkhoffii* and *B. henselae* bacteremia, as well as coinfection with both organisms, has also been reported in immunocompetent people with substantial arthropod and animal contact (3, 7). Based upon extensive travel histories, it is likely that both patients were exposed to a spectrum of animal reservoir hosts and transmission-competent insect vectors. Interestingly, patient 1, while traveling in the United States approximately 11 years prior to the diagnosis of EHE, developed severe cellulitis after experiencing numerous flea bites. Patient 2 reported a case of cellulitis, for which antibiotic treatment was prescribed, following bites from Blandford flies. Although the mode(s) and timing of transmission of the *Bartonella* spp. to these patients remain unknown, it is possible that both individuals were infected by insect bites that occurred years prior to the diagnosis of EHE.

Failure to consistently detect one or more *Bartonella* species in all three sample sets from a given batch of patient samples could have been related to a number of factors but most likely reflects variability in the level of bacteremia at the time of sample collection. In part, this observation could potentially be associated with a relapsing pattern of bacteremia, as has been reported in studies of cats experimentally infected by blood transfusion and in an experimental rodent model following inoculation of culture-grown bacteria (21, 32). Trench fever, caused by *B. quintana*, was historically referred to as Quintan fever due to the remitting febrile episodes that generally occurred at five-day intervals and most likely reflected a relapsing pattern of bacteremia. Clearly, the results from both patients and the boyfriend of patient 1 further illustrate current limitations associated with achieving a serological or molecular diagnosis of bartonellosis. In a few instances, *Bartonella* sp. DNA could be amplified by PCR; however, the amplicon could not be successfully sequenced, most likely due to a low DNA concentration. It is well recognized that *Bartonella* spp. are highly fastidious bacteria that require optimized culture conditions for isolation or, alternatively, molecular documentation of infection by PCR amplification and DNA sequencing (1–3). If *Bartonella* spp. induce a relapsing pattern of bacteremia in people such as occurs in experimentally infected animals, this factor would further complicate microbiological confirmation of bacteremia. Based upon results derived from patient 1, we now routinely test three sample sets obtained every other day over a 1-week collection period.

For patient 1, serological results were congruent with the *Bartonella* PCR results that were obtained as a result of sequential blood culture attempts; i.e., seroreactivity was demonstrated to each infecting *Bartonella* sp. eventually found in the patient's blood by PCR. In contrast, patient 2 and the boyfriend of patient 1 were both seronegative at the initial testing time points. Obtaining a batch of three serum samples within a period of several days has allowed us in most instances to document good overall reproducibility of serological results; however, there was an occasional unexplained variation (i.e., a 4-fold or greater variation in antibody titers) in IFA test results within a batch of serum samples. The extent to which this variability might occur in patients infected with *Bartonella* spp.

is unknown, as, in most instances, antibody testing is rarely repeated at such short testing intervals. Potentially, iatrogenic factors associated with sample collection, shipment, or storage interfered with the IFA testing procedure; alternatively, it is possible that *in vivo* or *in vitro* formation of antigen-antibody complexes resulted in lower antibody titers. Patient 2 spontaneously seroconverted to *B. vinsonii* subsp. *berkhoffii*, *B. henselae*, and *B. koehlerae* antigens during the course of the study, whereas *Bartonella* antibodies were detected in the boyfriend of patient 1 only following antibiotic treatment. Previously, we reported the lack of a detectable IFA antibody response in patients infected with *B. vinsonii* subsp. *berkhoffii* and *B. henselae* (4, 5). It is also not unusual in our experience for a seronegative patient to become seroreactive to *Bartonella* sp. antigens after receiving antibiotic therapy. Whether this observation reflects the movement of the bacteria from a sequestered intracellular location to an extracellular site where antigenic recognition of the bacteria by the host is enhanced or whether antigenic recognition is enhanced by antibiotic-induced injury or bacterial death is not known.

Repeated documentation of *B. koehlerae* in the boyfriend of patient 1 potentially supports the hypothesis of a common source of exposure (fleas or the pet cat, despite the negative test results) or the possibility of horizontal transfer of this bacterium by an as-yet-undetermined route(s). As the pet cat was seronegative and PCR negative (according to blood, serum, and oral swab test results), it was considered an unlikely source of infection. In contrast, *B. koehlerae* DNA was amplified and successfully sequenced from an oral swab sample provided by patient 1. Despite amplification of *B. koehlerae* DNA from the blood, oral swabs simultaneously obtained from the boyfriend of patient 1 on three consecutive days were PCR negative. Detection of *B. koehlerae* DNA in an oral swab does not confirm the presence of viable bacteria or, alternatively, if the bacteria are viable, whether oral transmission can actually occur. *Bartonella* DNA has previously been found in salivary samples from cats, dogs, and a bacteremic human patient with periodontal disease (1, 15). In addition, family clusters involving two or more patients infected with the same *Bartonella* spp. and strains have been recently reported (4, 5). Clearly, additional studies are required to better define modes of *Bartonella* transmission among vectors, animals, and people.

Based upon sequential serological and enrichment PCR testing results from patient 1 spanning a 22-month time period, therapeutic elimination of *Bartonella* infection in some EHE patients may be difficult to achieve. Similarly, *B. henselae* (strain SA2) was repeatedly amplified from blood, serum, or enrichment blood cultures from a veterinarian, despite extended courses of antibiotics (28). In conclusion, our findings do not confirm that *B. koehlerae*, *B. henselae*, and *B. vinsonii* subsp. *berkhoffii* are a cause of EHE. However, if the proposed association of *Bartonella* spp. with EHE were to be confirmed, it is plausible that eradicating the bacterial infection or interrupting *Bartonella*-induced angiogenic and proliferative cell signals could slow tumor progression and improve patient outcomes. As a novel WWTR1-CAMTA1 gene fusion in 17 EHE tumors was recently identified, the role of *Bartonella* and the bacteriophages contained in *B. henselae* and *B. vinsonii* subsp. *berkhoffii* as potential causes of recurrent chromosomal trans-

location involving CAMTA1 on 1p36.23 and WWTR1 on 3q25.1 should be investigated (17).

#### REFERENCES

1. Breitschwerdt, E. B., and R. G. Maggi. 2009. A confusing case of canine vector-borne disease: clinical signs and progression in a dog co-infected with *Ehrlichia canis* and *Bartonella vinsonii* ssp. *berkhoffii*. *Parasit. Vectors* 2(Suppl. 1):S3.
2. Breitschwerdt, E. B., R. G. Maggi, B. B. Chomel, and M. R. Lappin. 2010. Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. *J. Vet. Emerg. Crit. Care (San Antonio)* 20:8–30.
3. Breitschwerdt, E. B., et al. 2007. *Bartonella* species in blood of immunocompetent persons with animal and arthropod contact. *Emerg. Infect. Dis.* 13:938–941.
4. Breitschwerdt, E. B., R. G. Maggi, P. Farmer, and P. E. Mascarelli. 2010. Molecular evidence of perinatal transmission of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* to a child. *J. Clin. Microbiol.* 48:2289–2293.
5. Breitschwerdt, E. B., et al. 2010. *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* bacteremia in a father and daughter with neurological disease. *Parasit. Vectors* 3:29–37.
6. Breitschwerdt, E. B., et al. 2010. PCR amplification of *Bartonella koehlerae* from human blood and enrichment blood cultures. *Parasit. Vectors* 3:76–84.
7. Breitschwerdt, E. B., R. G. Maggi, W. L. Nicholson, N. A. Cherry, and C. W. Woods. 2008. *Bartonella* sp. bacteremia in patients with neurological and neurocognitive dysfunction. *J. Clin. Microbiol.* 46:2856–2861.
8. Breitschwerdt, E. B., R. G. Maggi, M. Varanat, K. E. Linder, and G. Weinberg. 2009. Isolation of *Bartonella vinsonii* subsp. *berkhoffii* genotype II from a boy with epithelioid hemangioendothelioma and a dog with hemangiopericytoma. *J. Clin. Microbiol.* 47:1957–1960.
9. Breitschwerdt, E. B., et al. 2011. Hallucinations, sensory neuropathy, and peripheral visual deficits in a young woman infected with *Bartonella koehlerae*. *J. Clin. Microbiol.* 49:3415–3417.
10. Chomel, B. B., H. J. Boulouis, S. Maruyama, and E. B. Breitschwerdt. 2006. *Bartonella* spp. in pets and effect on human health. *Emerg. Infect. Dis.* 12:389–394.
11. Dehio, C. 2008. Infection-associated type IV secretion systems of *Bartonella* and their diverse roles in host cell interaction. *Cell. Microbiol.* 10:1591–1598.
12. Dehio, C. 2004. Molecular and cellular basis of *Bartonella* pathogenesis. *Annu. Rev. Microbiol.* 58:365–390.
13. Dehio, C. 2003. Recent progress in understanding *Bartonella*-induced vascular proliferation. *Curr. Opin. Microbiol.* 6:61–65.
14. Diniz, P. P., et al. 2009. Co-isolation of *Bartonella henselae* and *Bartonella vinsonii* subsp. *berkhoffii* from blood, joint and subcutaneous seroma fluids from two naturally infected dogs. *Vet. Microbiol.* 138:368–372.
15. Duncan, A. W., R. G. Maggi, and E. B. Breitschwerdt. 2007. *Bartonella* DNA in dog saliva. *Emerg. Infect. Dis.* 13:1948–1950.
16. Duncan, A. W., R. G. Maggi, and E. B. Breitschwerdt. 2007. A combined approach for the enhanced detection and isolation of *Bartonella* species in dog blood samples: pre-enrichment liquid culture followed by PCR and subculture onto agar plates. *J. Microbiol. Methods* 69:3–281.
17. Errani, C., et al. 2011. A novel WWTR1-CAMTA1 gene fusion is a consistent abnormality in epithelioid hemangioendothelioma of different anatomic sites. *Genes Chromosomes Cancer* 50:644–653.
18. Florin, T. A., T. E. Zaoutis, and L. B. Zaoutis. 2008. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infection. *Pediatrics* 121:e1413–e1425.
19. Kempf, V. A., et al. 2005. Activation of hypoxia-inducible factor-1 in bacillary angiomatosis: evidence for a role of hypoxia-inducible factor-1 in bacterial infections. *Circulation* 111:1054–1062.
20. Kempf, V. A., et al. 2001. Evidence of a leading role for VEGF in *Bartonella henselae*-induced endothelial cell proliferations. *Cell Microbiol.* 3:623–632.
21. Kordick, D. L., and E. B. Breitschwerdt. 1997. Relapsing bacteremia after blood transmission of *Bartonella henselae* to cats. *Am. J. Vet. Res.* 58:492–497.
22. Lerut, J. P., et al. 2007. The place of liver transplantation in the treatment of hepatic epithelioid hemangioendothelioma: report of the European liver transplant registry. *Ann. Surg.* 246:949–957; discussion, 957.
23. Li, A., S. Dubey, M. L. Varney, B. J. Dave, and R. K. Singh. 2003. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J. Immunol.* 170:3369–3376.
24. Maeno, N., et al. 1999. Live *Bartonella henselae* enhances endothelial cell proliferation without direct contact. *Microb. Pathog.* 27:419–427.
25. McCord, A. M., S. I. Resto-Ruiz, and B. E. Anderson. 2006. Autocrine role for interleukin-8 in *Bartonella henselae*-induced angiogenesis. *Infect. Immun.* 74:5185–5190.
26. Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins. 1990. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. *N. Engl. J. Med.* 323:1573–1580.
27. Resto-Ruiz, S. I., et al. 2002. Induction of a potential paracrine angiogenic

- loop between human THP-1 macrophages and human microvascular endothelial cells during *Bartonella henselae* infection. *Infect. Immun.* **70**:4564–4570.
28. Sykes, J. E., L. L. Lindsay, R. G. Maggi, and E. B. Breitschwerdt. 2010. Human coinfection with *Bartonella henselae* and two hemotropic mycoplasma variants resembling *Mycoplasma ovis*. *J. Clin. Microbiol.* **48**:3782–3785.
29. Thomson, K. R., et al. 2011. Investigation of the safety of irreversible electroporation in humans. *J. Vasc. Interv. Radiol.* **22**:611–621.
30. Weiss, S. W., and F. M. Enzinger. 1982. Epithelioid hemangioendothelioma: a vascular tumor often mistaken for a carcinoma. *Cancer* **50**:970–981.
31. Yager, J. A., et al. 2010. Bacillary angiomatosis in an immunosuppressed dog. *Vet. Dermatol.* **21**:420–428.
32. Yamamoto, K., et al. 2002. Experimental infection of domestic cats with *Bartonella koehlerae* and comparison of protein and DNA profiles with those of other *Bartonella* species infecting felines. *J. Clin. Microbiol.* **40**:466–474.