Isolation of *Bartonella vinsonii* subsp *berkhoffii* genotype II from a boy with epitheloid hemangioendothelioma and a dog with hemangiopericytoma

Running Title: Vasoproliferative neoplasia and Bartonella infection

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Abstract:

In this report we describe isolation of Bartonella vinsonii subsp berkoffii genotype II from a boy with epitheloid hemangioendothelioma and a dog with hemangiopericytoma. These results suggest that B. vinsonii subsp berkoffii may cause vasoproliferative lesions in both humans and dogs.

Keywords: Vascular tumors, VEGF, infection, cancer
Case reports:

Case 1

A 13-year-old male presented with acute onset of severe right upper quadrant pain and hepatomegaly. The patient had a several month history of malaise and fatigue, which was attributed to mononucleosis (positive monospot test), but the boy was otherwise previously healthy. A CT scan identified large heterogeneously enhancing tumors throughout the liver. The patient had a mild microcytic anemia (Hb 12.5 g/dL; mcv 71.1) with very low serum iron (23 mcg/dL), normal iron binding capacity (292 mcg/dL) and low percent iron saturation (7.9%). C-reactive protein was elevated at 18 mg/L. Liver function tests were normal except for a mildly elevated gamma-glutamyl transpeptidase 76 IU/L (normal 11-63). A biopsy of the tumor was obtained laparoscopically. Histological analysis yielded a diagnosis of epithelioid hemangioendothelioma (EHE) with tumor cells staining strongly for endothelial markers CD31 and CD34. Subsequently and at different diagnostic time points, multiple venous blood samples, drawn following sterile skin preparation, were sent to the Intracellular Pathogens Research Laboratory (IPRL) at North Carolina State University for culture in *Bartonella* alpha Proteobacteria growth medicum (BAPGM) and 16S-23S intergenic spacer (ITS) PCR, as previously described (3, 4, 9). In addition, scrolls cut from the formalin preserved, paraffin-embedded block of the EHE tumor were tested for *Bartonella* spp. DNA using 16S-23S ITS PCR. *Bartonella vinsonii* subsp. *berkhoffii* genotype II was initially amplified and sequenced directly from a venous blood sample. (Table 1) Subsequently, *B. vinsonii* subsp. *berkhoffii* genotype II DNA was amplified and sequenced from the tissue block and again for a third time from a repeat BAPGM enrichment blood culture. Four distinct *B. vinsonii* subsp. *berkhoffii* genotypes have been characterized based upon defined insertion and deletion sequences within
the ITS region (5, 21). Only genotype II DNA was sequenced from the blood or tissues from this
boy. After consultation with infectious disease physicians, antibiotic treatment was instituted
comprising a triple drug therapy for twelve weeks duration, including oral doxycycline and
rifampin and, for the initial two weeks, intravenous gentamicin, which was replaced by oral
azithromycin for the remaining ten weeks. This regimen was chosen to achieve sufficient
intracellular concentrations of rifampin and azithromycin, serum concentrations of doxycycline
and to also invoke the putative bacteriocidal properties of aminoglycosides for the treatment of
Bartonella infection. Constitutional symptoms improved during the period of treatment and
improvement has continued during the subsequent year. The patient grew 5 cm and gained 7.7
kg. The patient reports rare, brief episodes of right upper quadrant pain, but remains otherwise
asymptomatic. Serial CT scans during the year following treatment have shown no change in the
size or number of tumors. The patient remains mildly anemic (Hb 12.7 g/dL). However, the C-
reactive protein concentrations (8 mg/L) and gamma-glutamyl transpeptidase (58 IU/dL)
dropped to near normal and normal values, respectively; other liver function test values have
remained in the normal range. For over one year following antibiotic administration, B. vinsonii
subsp. berkhoffii was not isolated and Bartonella spp. DNA was not amplified from five blood
culture samples (direct extraction, BAPGM enrichment liquid culture and agar plate subcultures
all negative) (Table 1). There has also been a progressive decrease in seroreactivity to B.
henselae, B. vinsonii subsp. berkhoffii genotype I and B. vinsonii subsp. berkhoffii genotype II
antigens, eventually achieving undetectable levels (Table 1).

Case 2

A 12-year-old female English sheepdog was referred to the North Carolina State University
Oncology Service for evaluation of a recurrent right antebrachial mass. Three years earlier, a
similar, large mass had been surgically resected by the referring veterinarian from the same
anatomic site. One year later, a smaller, approximately 4.0 to 6.0 cm mass was removed after
reoccurrence at the surgical excision site. Although no lameness was reported, the dog would
frequently lick the masses, presumably due to pain or local irritation. Historically, the dog had
been otherwise healthy.

At the time of referral, there were three distinct right foreleg masses measuring 0.5 x 0.3 x 1.5
cm on the medial surface of the right antebrachium, 3.0 x 3.0 x 1.5 cm on the cranial right
antebrachium and a 4.0 x 4.5 x 2.0 cm mass on the lateral right antebrachium. The masses were
discrete, non-tender, fixed and located between 2.0 and 5.0 cm distal to the olecranon. There
was also a firm 2.0 x 2.0 x 1.0 cm mass on the medial aspect of the right rear leg. Complete
blood count and serum biochemical profile values were normal. The dog was mildly proteinuric
(urine specific gravity 1.030, 2+ urine protein, negative urine sediment). Aspiration cytology of
the right antebrachial and right medial thigh masses identified sheets or clusters of fusiform cells
with a high nuclear to cytoplasmic ratio, basophilic cytoplasm and prominent nucleoli, consistent
with a mesenchymal neoplasm. On thoracic radiographs, there was no evidence of pulmonary
metastases. Core biopsies were obtained from the antebrachial and medial thigh masses.
Histopathology of the masses identified densely-packed and interweaving streams and
fingerprint whorls of plump, elongated spindle-shaped cells separated by a scant fibrillar stroma,
which contained numerous small blood vessels. Neoplastic cells occasionally whorled around
small blood vessels. The cells contained scant to moderate amounts of amphophilic, weakly
fibrillar cytoplasm, oval nuclei with finely stippled chromatin, one to two nucleoli per cell and
infrequent mitoses. There was mild anisokaryosis and anisocytosis and rare multinucleated cells.
The location, morphology and recurrent nature of these tumors were most consistent with hemangiopericytoma.

This dog was referred to our teaching hospital in 1995, two years after the NCSU-IPRL had made the first isolate of \textit{B. vinsonii} subsp. \textit{berkhoffii} (ATCC type strain 93-CO-1) genotype I from a dog with endocarditis (1). Using \textit{B. vinsonii} subsp. \textit{berkhoffii} genotype I as the antigen source, indirect immunofluorescent antibody titers (n=4) were consistently 1:256 over the next 3 months and a \textit{Bartonella} spp. was isolated by lysis centrifugation blood culture. Retrospectively, the strain was determined to be \textit{B. vinsonii} subsp. \textit{berkhoffii} genotype II, based upon sequencing the 16S-23S intergenic spacer region in 2008, using a previously described approach (21).

Three months after the initial oncology consultation the owners elected surgery. Mild basophilia (200 cells/µl, normal less than 100 basophils/µl) was the only hematological abnormality and there were no serum biochemical abnormalities. The medial thigh mass was totally resected and the three antebrachial masses were partially resected. The owner declined radiation therapy for the antebrachial mass. The dog was treated with enrofloxacin for 6 weeks at which time the \textit{B. vinsonii} subsp. \textit{berkhoffii} antibody titer was 1:128 and a lysis centrifugation blood culture was negative. Also in 2008, \textit{B. vinsonii} subsp \textit{berkhoffii} genotype II DNA was amplified and sequenced from the paraffin embedded biopsy of the antebrachial hemangiopericytoma; the block had been stored for 13 years.

In this report, infection with \textit{B. vinsonii} subsp. \textit{berkhoffii} genotype II is described in a boy with epitheloid hemangioendothelioma (EHE) and a dog with hemangiopericytoma. Neoplastic
recurrence at the same anatomic location over a 3-year period, in conjunction with the temporal association of *B. henselae* and *B. quintana* with vasoproliferative lesions in HIV-infected humans (25, 26), initiated our efforts to determine if the dog was infected with a *Bartonella* spp. The boy was cultured after a literature search by an attending physician revealed a well-established association between *Bartonella* spp. infection, vascular endothelial growth factor (VEGF) induction and angioproliferative disease (6, 7, 13, 27). An association of increased tissue VEGF levels with the formation of hemangioendotheliomas lent credence to the hypothesis of a potential causal association between Bartonella infection and hemangioendothelioma (18, 30).

Dogs and humans infected with *Bartonella* spp. can develop similar disease manifestations and pathological lesions, including prototypical vasoproliferative lesions such as bacillary angiomatosis and peliosis hepatis (2, 6). In humans, *Bartonella henselae* and *Bartonella quintana* cause cutaneous vasoproliferative lesions (bacillary angiomatosis) and parenchymal vasoproliferative lesions of the liver, spleen (bacillary peliosis) and less frequently other tissues, particularly in human immunodeficiency virus (HIV) infected patients (25, 26). The NCSU-IPRL recently cultured *B. vinsonii* subsp. *berkhoffii* genotype I from a dog with bacillary angiomatosis (Yager J, Breitschwerdt E et al in preparation) and previously *B. henselae* DNA was amplified from the liver of a dog with peliosis hepatis (14). Long-lasting (months to years) intravascular infection with *B. henselae* has been documented in naturally and experimentally-infected cats (16-17) and persistent intravascular bacteremia for 18 months with *B. vinsonii* subsp. *berkhoffii* genotype II was reported in a naturally-infected healthy pet dog (15). More recently, persistent infection with *B. vinsonii* subsp. *berkhoffii* and *B. henselae*, as well as co-
infection with both organisms, has been reported in immunocompetent people with substantial arthropod and animal contact (3, 4). The seemingly unique capability of bacteria of the genus *Bartonella* to invade and induce long lasting intra-erythrocytic and intra-endothelial infections, in conjunction with the ability of at least three *Bartonella* spp. (*B. henselae*, *B. quintana* and *Bartonella 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 other vascular tumors (7, 8, 12).

*Bartonella vinsonii* subsp. *berkhoffii* genotype I was isolated for the first time from a dog with epistaxis, recent-onset seizures and endocarditis in 1993 (1). Subsequently, three additional genotypes (designated II-IV), all of which have been implicated as a cause of endocarditis in dogs, were described based upon sequence differences in the *Bartonella* 16S-23S intergenic spacer region and the Pap31 gene (5, 21). Genotypes I, II and III have also been implicated in humans with vascular infections (3, 4, 29). Although seemingly well-adapted on an evolutionary basis to induce persistent infection in canine reservoir hosts (dogs, foxes and coyotes), *B. vinsonii* subsp. *berkhoffii* has only rarely been isolated from pet dogs (2, 6). In pet dogs, both seroprevalence studies and blood culture isolation studies indicate infrequent exposure to or active infection with any of the four *B. vinsonii* subsp. *berkhoffii* genotypes; whereas infection is more frequent in coyotes and feral dog populations (6, 21). Although a source of infection was not determined for either case, the dog in this case report was from a rural area and had experienced recurrent ectoparasite exposures. The boy had a tick bite roughly one year prior to the diagnosis of EHE, spent summers in heavily wooded areas and lived in a suburb where
coyote sightings are common. Coyotes are likely a reservoir host for *B. vinsonii* subsp. *berkhoffii* genotype II and tick transmission of this subspecies has been proposed on the basis of epidemiological evidence (2, 6). In regard to human infections, there is one case of endocarditis and 8 cases in which *B. vinsonii* subsp. *berkhoffii* was isolated or sequenced from blood cultures obtained from immunocompetent people with arthritis, fatigue, neurological or neurocognitive abnormalities (3, 4, 29). Four of these eight *B. vinsonii* subsp. *berkhoffii* infected individuals were co-infected with *B. henselae* and *B. vinsonii* subsp. *berkhoffii*, of which genotype II was sequenced from all but one person, who was infected with genotype I. We were unable to detect DNA evidence of *B. henselae* infection using species-specific primers in samples from the boy or the dog. Therefore, isolation of the same bacterial genotype from vascular tumors occurring in a dog and a human suggests that *B. vinsonii* subsp. *berkhoffii* may play a role in the development of some vasoproliferative tumors. Over a decade separated the efforts to isolate *B. vinsonii* subsp. *berkhoffii* from the dog and the human patient in this study. In the intervening period, there were important advances in the microbiological isolation and the molecular detection of *Bartonella* spp. in blood and tissues from immunocompetent individuals, which continue to facilitate a redefinition of the pathogenic role of this genus in animals and humans (3, 4, 9).

In all mammals, including dogs and humans, endothelial cells appear to be an important target cell following direct or vector-borne transmission of a *Bartonella* spp. (6-8). Based upon *in vitro* infection of human endothelial cell lines, *B. henselae* has been shown to induce angiogenesis and endothelial cell proliferation (20). *Bartonella* spp. also subvert many functions of human endothelial cells, including the induction of mitogenic and pro-inflammatory genes, cytoskeletal
rearrangements and suppression of endothelial cell apoptosis (7, 8). Clinically, the resulting vascular proliferation induces tumor-like lesions (verruga peruana, bacillary angiomatosis, peliosis hepatis), especially in immunocompromised individuals (2, 6, 7). VEGF is an important mediator of tumor angiogenesis, and its production has been specifically induced by *B. henselae* in vitro (13, 23). In addition, increased VEGF levels were found in tissues from patients with bacillary angiomatosis and peliosis hepatis (23). Recently it has been shown that infection of human endothelial cells by *B. henselae* resulted in IL-8 production and upregulation of IL-8 receptors CXCR2 (23). IL-8 promotes angiogenesis through enhanced endothelial cell survival and enhanced vascular proliferation (13, 19, 23). The results of this study suggest that *B. vinsonii* subsp. *berkhoffii*, a *Bartonella* spp. that appears to have co-evolved with canines (2, 6) may also contribute to vasoproliferative lesions in dogs and human beings, potentially by enhancing cell proliferation in conjunction with inhibition of apoptosis. Whether *Bartonella*-triggered vasoproliferation is a pathogenic strategy used by these bacteria to expand a specific host cell habitat (the endothelial cell), is currently unknown (12, 24).

Hemangiopericytoma (HPC) is a vascular neoplasm thought to originate from pericytes, capillary subendothelial lining cells predominantly found in distal extremity vessels (11, 22). As described in this report, these tumors in dogs are generally subcutaneous and have a predilection for involving the limbs (11, 22). HPCs are most often found in adult dogs, there is no sex predilection, local recurrence is common and metastasis is rare (11, 22) EHE is a rare vascular neoplasm having a malignant potential between benign hemangioma and angiosarcoma. First described by Weiss in 1982 (30), EHE is characterized by positive immunostains for endothelial antigens CD34 and Factor VIII. EHE typically presents with various combinations of hepatic,
pulmonary, cutaneous or bony disease. The natural history is extremely unpredictable and there
is a substantial inter-patient variability in disease progression. Intervals of rapid growth are
frequently interrupted by long periods of quiescence. EHE generally is unresponsive to standard
cytotoxic chemotherapy and radiation therapy. Despite anecdotal reports of success with anti-
angiogenics, there have been no clinical trials of this approach. Surgery is considered the
treatment of choice in hepatic disease, where options include resection for localized disease or
transplant for the more common presentation of multilobar, multicentric liver involvement, as
would be the case for the boy in this report. Two large studies indicate a high percentage of long-
term survivors following liver transplant, even in the presence of metastases (18, 28). If the
proposed association of Bartonella spp. with EHE were 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.

On a comparative medical basis, dogs and humans infected with Bartonella spp. can develop
similar disease manifestations, including endocarditis, granulomatous lymphadenitis,
granulomatous hepatitis, bacillary angiomatosis, peliosis hepatis, seizures and arthritis (2, 6).
Therefore medical information generated in one species (dogs or human beings) can prove
beneficial while attempting to characterize the role of Bartonella spp. as a pathogen in the
comparable species. As is true of many other infectious diseases, a “One Medicine” approach to
the current clinical and research understanding of canine and human bartonellosis has proven
beneficial for the health care of animals and human patients (10, 31). During the past decade,
researchers have provided substantial evidence to support a role of infectious agents including
bacteria, viruses, mycoplasma and protozoa as cofactors in the development of cancer in humans.
Substantial epidemiologic and microbiological research is needed to test the potential causal relationship of *Bartonella* spp. with EHE and HPC and to determine whether members of the genus *Bartonella* will be added to the list of oncogenic infectious agents in the future.

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The dog *B. vinsonii* subsp. *berkhoffii* genotype II isolate reported in this study was obtained by Brandee Pappalardo while a Ph. D. student in the Intracellular Pathogens Research Laboratory at North Carolina State University. We thank Julie Bradley for providing *Bartonella* spp. serological test results, Tonya Lee for editorial assistance and the many clinicians who facilitated the care of these patients.
References:


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Söderdahl, R. Steininger, A. Wettergren, V. Mazzaferro, Y. P. Le Treut, and V. Karam.


Table 1. Serological, PCR and culture results for a 13-year-old male with a hepatic epitheloid hemangioendothelioma and *Bartonella vinsonii* subsp. *berkhoffii* infection.

<table>
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<th>Date/Sample</th>
<th>B. henselae</th>
<th>Bvb I</th>
<th>Bvb II</th>
<th>Direct Extraction</th>
<th>Enrichment Culture</th>
<th>Plate Isolate</th>
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<td>64</td>
<td>16</td>
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<td>64</td>
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*Bvb* = *Bartonella vinsonii* subsp. *berkhoffii*, *Denotes 16S-23S ITS DNA sequence result that defines genotypes I-IV. NA = Not applicable, NT = Not tested serologically.