

Bartonella henselae and *Bartonella elizabethae* as Potential Canine Pathogens

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***Bartonella henselae* or *Bartonella elizabethae* DNA from EDTA-anticoagulated blood samples obtained from four dogs was amplified and sequenced. The results showed that *B. elizabethae* should be added to the list of *Bartonella* species (i.e., *B. vinsonii* subsp. *berkhoffii*, *B. henselae*, and *B. clarridgeiae*) that are currently recognized as infectious agents in dogs. Furthermore, these results may have potential zoonotic implications, particularly if dogs can serve as a previously unrecognized reservoir for *B. henselae*. Although the clinical relevance of these observations remains to be determined, it is possible that molecular diagnostic techniques such as PCR may help to implicate a spectrum of *Bartonella* spp. as a cause of or a cofactor in chronic canine and human diseases of poorly defined causation.**

The genus *Bartonella* comprises at least 15 species of gram-negative, hemotropic, short, rod-shaped bacteria that have been recognized as agents in emerging diseases of humans and other mammalian species. Historically, *Bartonella* species have been associated with important epidemics, such as the outbreaks of Oroya fever or verruga peruana, caused by *B. bacilliformis*, in the Andes Mountains in 1871 and trench fever, caused by *B. quintana*, during World War I (14). Transmission of all *Bartonella* spp. is believed to be vector borne, i.e., by endemic arthropods (4). More recently, *Bartonella* spp. have been associated with fever of unknown origin (12, 26), typical and atypical cat scratch disease (with or without hepatosplenic involvement), bacillary angiomatosis, peliosis hepatis (17), endocarditis (7, 24), and ocular lesions such as neuroretinitis in human patients (9, 15, 25, 27). Both immunocompetent and immunocompromised individuals have developed a similar spectrum of disease manifestations associated with *Bartonella* infections, and most, but not all, have had documented contact with a known mammalian reservoir species or an arthropod vector.

In 1993, blood cultures from a human with valvular endocarditis grew a fastidious gram-negative bacterium. DNA from the isolate showed 98.9% similarity to *Bartonella* (previously *Rochalimaea*) species (7). The new strain was designated *Rochalimaea elizabethae* (now *Bartonella elizabethae*), and the DNA sequence was recorded in GenBank under accession no. L35103 (7). To our knowledge, *B. elizabethae* has only been isolated from this patient and from rats (*Rattus norvegicus*) (10). In contrast, *B. henselae* has been isolated from cats throughout the world and occasionally from people with fever of unknown origin (12, 26) or endocarditis (24) or from homeless patients (5). Data on the prevalence of *B. henselae* in dogs are limited. Two studies (1, 8) reported *B. henselae* seroprevalences of 6.5 and 3%, respectively, in their dog populations.

However, bacteria were not isolated and PCR was not performed. A study from Japan reported a *B. henselae* seroprevalence of 7.7% (4 of 52 dogs) and positive *B. henselae* PCR results from peripheral blood, nail clippings, and oral swabs; however, these amplicons were never sequenced (M. Tsukahara, H. Tsuneoka, H. Iino, I. Murano, H. Takahashi, and M. Uchida, Letter, Lancet **352**:1682, 1998).

During the last decade, studies with dogs have provided evidence that *B. vinsonii* subsp. *berkhoffii* can cause endocarditis (2, 3), granulomatous lymphadenitis, and granulomatous rhinitis (21). In contrast, *B. henselae* DNA has been amplified and sequenced from only one dog that died of peliosis hepatis (16). To date, *B. henselae* is the only *Bartonella* sp. that has been implicated as a cause of peliosis hepatis in dogs (16) and in humans (17). In 1993, Breitschwerdt et al. isolated a novel *Bartonella* species, eventually named *B. vinsonii* subspecies *berkhoffii*, from the blood of a dog with vegetative valvular endocarditis (3, 18). Subsequently, *B. vinsonii* subsp. *berkhoffii* DNA was amplified and sequenced from blood and/or heart valves of three additional dogs with endocarditis (2). Epidemiologic evidence suggested tick exposure as a risk factor for *Bartonella* infection in dogs (22). Recently, 5 of 18 dogs (28%) with endocarditis, including three dogs infected with *B. vinsonii* subsp. *berkhoffii*, examined at the Veterinary Medicine Teaching Hospital, University of California, Davis, were seroreactive to *Bartonella* spp. antigens and PCR positive for *Bartonella* spp. (B. Chomel, Abstr. Second Workshop on Comparative Medicine, Lyon, France, p. 61, 2001). These results prompted a study of archived cardiac valves from U.S. Army working dogs, which led to the addition of six more PCR-positive cases of *B. vinsonii* subsp. *berkhoffii* to the literature (B. Chomel, Abstr. Second Workshop on Comparative Medicine, Lyon, France). Most recently, *B. clarridgeiae*, which was first described after its isolation from a cat in 1995, was amplified and sequenced from the heart valve of a young Boxer dog with vegetative endocarditis at the University of California, Davis (6).

Thus, although *B. vinsonii* subsp. *berkhoffii* was the first *Bartonella* sp. to be identified as a pathogen in dogs, it is increasingly evident that *B. henselae*, *B. clarridgeiae*, and potentially

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other *Bartonella* spp. may also cause illness in dogs. Furthermore, infection with these bacteria appears to induce disease manifestations, such as endocarditis, peliosis hepatis, and granulomatous disease, that are similar to those observed in both dogs and humans. The purpose of this report is to describe cases involving three dogs infected with *B. henselae* and one dog infected with *B. elizabethae* for which there were no compatible signs or diagnostic evidence of endocarditis or liver disease, manifestations previously associated with *Bartonella* spp. infection in dogs. We are not aware of other reports describing the molecular detection of *B. henselae* or *B. elizabethae* DNA from the blood of sick dogs. Future studies should be aimed at elucidating the pathogenic significance of infection of dogs with various *Bartonella* spp.

CASE REPORTS

Case 1. A 10-year-old, male, castrated Rottweiler (dog 1) from Oxford, N.C., was referred to the North Carolina State University Veterinary Teaching Hospital (NCSU-VTH), Raleigh, N.C., with a 5-month history that included intermittent lethargy, anorexia, oral ulcerations, bilateral mucopurulent nasal discharge, and significant weight loss (approximately 30 kg over 8 months). Treatment with several different antibiotics resulted in only partial improvement of clinical signs. On presentation, the dog was quiet, alert, responsive, weighed 32.4 kg, and had a rectal temperature of 39.0°C. There was mild to moderate lymphadenopathy. Abdominal palpation revealed a painful, 3- by 5-cm abdominal mass, and an ophthalmic examination revealed chorioretinitis.

Hematologic abnormalities included thrombocytosis (platelet count, 453,000/ μ l; reference range, 181,000 to 350,000/ μ l), mild lymphopenia (lymphocyte count, 987/ μ l; reference range, 1,000 to 5,000/ μ l), and eosinophilia (eosinophil count, 846/ μ l; reference range, 100 to 750/ μ l). Thoracic radiographs were unremarkable, and abdominal radiographs were potentially consistent with partial intestinal obstruction. Exploratory surgery revealed a toothpick penetrating the wall of the small intestine. Resection and anastomosis were completed with no complications. Aspiration cytology from several enlarged peripheral lymph nodes identified reactive lymphoid hyperplasia with high numbers of mast cells and eosinophils. Serum from the dog was reactive to *B. vinsonii* subsp. *berkoffii* antigens at a reciprocal titer of 256. *B. henselae* DNA was amplified and sequenced from a peripheral blood sample. Enrofloxacin treatment (5 mg/kg of body weight; administered orally [p.o.] twice a day [BID]) and ampicillin (22 mg/kg; p.o., BID) was dispensed for six weeks for a presumptive diagnosis of bartonelosis. Ten weeks later, the dog was alert, active, and eating normally. The chorioretinitis had also resolved. Thrombocytosis (platelet count, 418,000/ μ l; reference range, 181,000 to 350,000/ μ l) persisted, but the lymphopenia and eosinophilia had resolved. The lymph node cytopathology was again consistent with lymphoid hyperplasia, without accompanying mast cells or eosinophils. Convalescent-phase serum antibodies to *B. vinsonii* subsp. *berkoffii* or *B. henselae* antigens were no longer detected.

Case 2. A 5-year-old, female, spayed Great Dane (dog 2) from Fayetteville, N.C., was referred to the NCSU-VTH with a history of refractory thrombocytopenia (platelet counts rang-

ing from 37,000 to 91,000/ μ l; reference range, 175,000 to 300,000/ μ l) and a previous serologic diagnosis of ehrlichiosis, which was treated with a protracted course of doxycycline. During the 19-month period of illness, the dog had lost approximately 16 kg. Physical examination was unremarkable. Hematologic abnormalities included mild lymphopenia (lymphocyte count, 930/ μ l; reference range, 1,000 to 5,000/ μ l), eosinophilia (eosinophil count, 930/ μ l; reference range, 100 to 750/ μ l), and marked thrombocytopenia (platelet count, 9,000/ μ l; reference range, 181,000 to 350,000/ μ l). Urine abnormalities included hematuria and proteinuria, with a urine protein-to-creatinine ratio of 1.5 and a specific gravity of 1.016. Antibodies to *B. vinsonii* subsp. *berkoffii* or *B. henselae* antigens were not detected. A blood culture maintained for 6 weeks failed to grow bartonella or other fastidious bacteria, and *Bartonella* DNA was not detected by PCR. Due to the persistently low platelet counts, the dog was treated with prednisone at immunosuppressive doses (1 mg/kg; p.o., BID) for a presumptive diagnosis of immune-mediated thrombocytopenia and concurrently with enrofloxacin (5 mg/kg; p.o., every 24 h), ampicillin (22 mg/kg; p.o., BID), and doxycycline (5 mg/kg; p.o., BID) for 2 weeks while awaiting blood culture results. During the next 4 weeks, the platelet count normalized (190,000/ μ l) and the dog's appetite began to increase while the animal received antibiotics and corticosteroids. Following the course of antibiotics, the corticosteroids were tapered and another EDTA blood sample was submitted for PCR testing. *B. henselae* DNA was amplified and sequenced from this sample, which was obtained 3 months after the initial presentation to our hospital. Six months later, the dog remained healthy without taking any medications; however, the most recent platelet count was only 92,000/ μ l.

Case 3. An 8-year-old, male, castrated Labrador retriever (dog 3) from Shelby, N.C., was examined at an emergency clinic because of acute onset of ataxia. The dog was hypermetric, with conscious proprioception deficits in the hind limbs. Hematologic abnormalities included mild leukocytosis (leukocyte count, 16,700/ μ l; reference range, 4,000 to 15,500/ μ l) with mature neutrophilia (neutrophil count, 14,028/ μ l; reference range, 2,060 to 10,600/ μ l), lymphopenia (lymphocyte count, 668/ μ l; reference range, 690 to 4,500/ μ l), and monocytosis (monocyte count, 1,837/ μ l; reference range, 0 to 840/ μ l). The reciprocal titer to *B. vinsonii* antigens was 128. Antibodies against *B. burgdorferi*, *Babesia canis*, *Rickettsia rickettsii*, or *Ehrlichia canis* antigens were not detected. Azithromycin (10 mg/kg; once a day for 5 days, then every other day for 40 days) was used for treatment of *Bartonella* infection. Three months later, the dog continued to have difficulty ambulating and was referred to a neurologist for evaluation of possible cauda equina syndrome. A myelogram and epidurogram revealed no compressive lesions in the cervical, thoracic, or lumbar spine; however, there was attenuation of the contrast column around the level of L5-L6, suggestive of an inflammatory lesion. There was a mild increase in cerebrospinal fluid protein content (59 mg/dl; normal range, 0 to 48 mg/dl), with no white blood cells. Prior to initiation of antibiotics, a serum sample was negative for antibodies to *B. vinsonii* and *B. henselae* antigens; however, *B. henselae* DNA was amplified by PCR. Treatment consisted of a 6-week course of amoxicillin-clavulanic acid (13 mg/kg; p.o., BID) and azithromycin (10 mg/kg; p.o., every 24 h).

Case 4. An 8-year-old male Shetland Sheepdog (dog 4) from Annapolis, Md., was examined by a veterinarian after a 2-month history of lethargy, decreased appetite, weight loss, and occasional vomiting of undigested food. On physical examination, mucous membranes were pale, the dog was very thin, and breathing was labored. Hematologic abnormalities included anemia (red blood cells, 3.97 M/liter [reference range, 5.20 to 8.33 M/liter]; hematocrit, 25.8% [reference range, 36 to 60%]; hemoglobin, 8.4 g/dl [reference range, 12.4 to 19.2 g/dl]), leukocytosis (leukocyte count, 26,900/ μ l; reference range, 5,100 to 17,600/ μ l), mature neutrophilia (neutrophil count, 21,800/ μ l; reference range, 1,900 to 14,200/ μ l), monocytosis (monocyte count, 1,900/ μ l; reference range, 0.0 to 1.4/ μ l), and eosinophilia (eosinophil count, 1,900/ μ l; reference range, 0.0 to 1,300/ μ l). Biochemical abnormalities included azotemia (blood urea nitrogen, 71 mg/dl; [reference range, 3 to 28 mg/dl]; creatinine, 2.8 mg/dl [reference range, 0 to 1.5 mg/dl]) and elevated amylase activity (3,702 IU/liter; reference range, 26 to 2,150 IU/liter). The urine specific gravity was 1.017, with 3+ protein, 2+ blood, 1+ bilirubin, 3 to 5 white blood cells per high-power field, 3 to 5 red blood cells per high-power field, and 2+ cocci, with amorphous crystals and granular casts. Serum and EDTA blood samples were submitted to the Vector Borne Disease Diagnostic Laboratory at North Carolina State University for diagnostic testing. During the next 2 days, the dog's condition deteriorated rapidly, and he died before results were obtained. The EDTA blood sample was later found to contain *B. elizabethae* DNA by PCR amplification and sequencing. Antibodies to *B. burgdorferi*, *E. canis*, or *B. canis* antigens were not detected, whereas the reciprocal titers to *B. vinsonii* subsp. *berkhoffii* and to *R. rickettsii* were 64 and 512, respectively.

MATERIALS AND METHODS

Case selection. The samples from the dogs described in this report represent the only blood samples tested by PCR in the Vector Borne Disease Diagnostic Laboratory at North Carolina State University between September 1999 (introduction of the primers described below) and November 2001 from which *B. henselae* or *B. elizabethae* DNA could be amplified. To our knowledge, these dogs represent the first reported cases of such infections, as identified by PCR amplification and sequence analysis from EDTA blood samples. We retrospectively analyzed each case record for signalment, anamnesis, physical exam findings, and other diagnostic test results to present a brief summary of relevant findings. As dogs 3 and 4 were not examined at NCSU-VTH, medical records were obtained from the attending veterinarians for review. For all cases, additional information was obtained when necessary by further communication with the veterinarians or the owners.

Serology. All dogs were tested at least once for antibodies against *B. vinsonii* subsp. *berkhoffii* and *B. henselae* by using previously described methods (22). Antigens used in testing for *B. elizabethae* antibodies were not available in our laboratory.

PCR. Blood obtained aseptically from the jugular vein was placed in tubes containing EDTA. DNA was extracted with a commercially available QIAamp blood kit (QIAGEN, Chatsworth, Calif.). Sample processing, DNA extraction, and PCR amplification were performed in separate rooms to avoid PCR contamination. Positive (tissue culture-grown *B. henselae*) and negative (reagent and uninfected EDTA blood) controls were processed with each patient sample. DNA was extracted from 200 μ l of blood. The PCR methods used for sample analysis amplified a fragment of the 16S-23S rRNA intergenic region with primers complementary to conserved sequences among *Bartonella* species that are known to infect mammals (13). The advantages of this test are that it can be performed as a one-step process and that it facilitates speciation according to band size after fractionation by gel electrophoresis (12). The primers used, 5'-CTCTTCTTCAGATGATGATCC-3' and 5'-AACCAACTGAGCTACAA GCCCT-3', resulted in amplified products of the expected size, 163 bp (*B.*

henselae) and 232 bp (*B. elizabethae*), as previously described (13). In each case, the amplicon was then sequenced using a SequiTherm EXCEL II DNA sequencing kit-LC as recommended by the supplier (Epicentre Technologies, Madison, Wis.). The sequencing reactions were performed as follows: 2 min at 92°C, followed by 30 amplification cycles (30 s at 92°C, 15 s at 55°C, and 30 s at 72°C) (Hybaid PCR Express). The sequencing reactions were analyzed by polyacrylamide gel electrophoresis (3.75%) on a LI-COR 4200 automated DNA sequencer.

Culture. At the time of the first evaluation at NCSU-VTH, blood samples from dogs 1 and 2 were submitted for *Bartonella* culture and were processed using previously described techniques (17).

RESULTS

At the time of initial examination, dogs 1, 3, and 4 had serum antibodies that were reactive against *B. vinsonii* antigens at reciprocal titers of 256, 128, and 64, respectively. Following administration of antibiotics, *B. vinsonii* subsp. *berkhoffii* antibodies were no longer detected in convalescent samples from dogs 1 and 3. Attempts to culture bartonella from the blood of dogs 1 and 2 were unsuccessful, potentially due to prior administration of antibiotics.

PCR amplification of DNA from EDTA-anticoagulated blood samples, followed by DNA sequencing as described above, resulted in the identification of *B. henselae* 16S-23S rDNA in dogs 1 to 3, with amplicons that had complete homology to the *B. henselae* sequence (GenBank accession no. L35101). When sequenced, the amplicon from dog 4 contained 16S-23S ribosomal DNA with complete homology to the sequence for *B. elizabethae* (GenBank accession no. L35103) (12).

DISCUSSION

To our knowledge, the description of these dogs represents the first report in which *B. henselae* or *B. elizabethae* DNA was amplified from canine peripheral blood samples. These results mean that *B. elizabethae* should be added to the list of *Bartonella* species (i.e., *B. vinsonii* subsp. *berkhoffii*, *B. henselae*, and *B. clarridgeiae*) that are currently recognized as infectious agents in dogs and provide additional evidence supporting a pathogenic role for *B. henselae* in dogs. Furthermore, these results may have potential zoonotic implications, particularly if dogs can serve as a previously unrecognized reservoir for these organisms. The frequency with which dogs infected with cat (*B. henselae*) or rodent (*B. elizabethae*) *Bartonella* organisms develop chronic occult infection is unknown. Previous efforts to detect *B. henselae* antibodies in flea-infested dogs have not been successful (22); however, studies involving healthy dogs from Hawaii, England, and Japan have detected *B. henselae* antibodies in a small number of dogs. The PCR test used in the evaluation of these four dogs has been described previously (13). This one-step PCR procedure was designed to facilitate species-specific differentiation based upon amplicon size without the need for further confirmatory testing or sequencing. However, because of the experimental nature of this assay in our laboratory and the unexpected results obtained in these four cases, amplicon specificity was confirmed by sequencing. Sequencing was also deemed necessary because this PCR technique, as originally described (13), has resulted in the occasional amplification of canine DNA in our laboratory. In these instances, the amplicon size was the same as the size expected

for *Bartonella* DNA, indicating that technical aspects of the assay need to be perfected. One limitation of PCR techniques in general is a lack of sensitivity in the analysis of small sample volumes or analysis of samples that contain low numbers of bacteria in blood or other tissues. If canine infections with feline *B. henselae* and *B. clarridgeiae* (7, 16; this report) or rodent *B. elizabethae* (10) strains result in a very low level of bacteremia, then even the enhanced sensitivity associated with PCR may not be sufficient to allow consistent detection of chronic infection in dogs. In this regard, surgical illness in dog 1, corticosteroids in dogs 2 and 3, and the terminal stages of illness in dog 4 may have facilitated PCR amplification of *B. henselae* and *B. elizabethae* DNA from the blood of these dogs. Confirmation of our results was achieved by repeat testing using the same samples and the same PCR technique. It is unlikely that false positive results were obtained in all four cases, given that all samples were run with concurrent positive and negative controls. In addition, these were the only positive samples found to contain *B. henselae* and *B. elizabethae* during the study period.

The clinical relevance derived from the microbiologic evaluation of these dogs is difficult to infer due to the small number of cases and the wide variation in historical, clinical, hematologic, and biochemical abnormalities. Interpretation is further complicated by the concurrent presence of other diseases (e.g., an intestinal foreign body in dog 1, potential immune-mediated thrombocytopenia in dog 2, and renal failure in dog 4). However, it is important to note several features shared among these dogs which may support a primary or opportunistic pathogenic role for *B. henselae* and *B. elizabethae*. Nonspecific clinical abnormalities, such as severe weight loss, protracted lethargy, and anorexia, in addition to a chronic disease course were common to all four cases. Evidence suggesting that *B. vinsonii* subsp. *berkhoffii* is a cause of chronic infections in dogs is well documented in experimentally infected beagles (20) and is further supported by the chronic disease course observed in naturally infected dogs (2, 3). Experimental infection of dogs with *B. vinsonii* subsp. *berkhoffii* causes immune suppression that is characterized by decreased numbers of CD8⁺ T lymphocytes, defects in monocytic phagocytosis, and impaired antigen presentation within lymph nodes (20), which could potentially predispose infected dogs to the development of autoimmune or immune-mediated disease manifestations. Although these nonspecific clinical findings are common in other occult infections, as well as noninfectious diseases, these changes are also commonly associated with the terminal stages of conditions of unknown or poorly defined etiology, such as glomerulonephritis, idiopathic immune-mediated disorders, and neoplasia. It is possible that advanced diagnostic techniques, such as PCR, may help to implicate occult infections with *Bartonella* spp. as a cause of or a cofactor in chronic diseases of poorly defined causation.

The most obvious hematologic and biochemical abnormalities observed in these four dogs included eosinophilia, monocytosis, alterations in platelet numbers, and elevations in serum amylase values. During the course of illness, three dogs had monocytosis, which is most commonly associated with acute or chronic tissue inflammation. Monocytosis was a common finding in a previous report that implicated *B. vinsonii* subsp. *berkhoffii* and related alpha *Proteobacteria* as a cause of cardiac

arrhythmias, endocarditis, or myocarditis in dogs (2). Thrombocytosis was documented in dogs 1, 3, and 4, while dog 2 had persistent and presumably immune-mediated thrombocytopenia. This persistent thrombocytopenia in dog 2 was attributed to immune-mediated platelet destruction, and the dog responded to immunosuppressive corticosteroid therapy. Thrombocytopenia, generally mild in degree, is the most consistent hematologic abnormality in humans chronically infected with *B. quintana* (5, 23); however, thrombocytosis has also been reported in humans infected with *B. henselae* (19).

Three dogs had increased amylase values within 1 year of diagnosis of *Bartonella* infection, while only one dog had an increased amylase value at the time *Bartonella* DNA was detected by PCR. However, the increased serum amylase value in this dog may have been secondary to early renal insufficiency. The role of *Bartonella* infections in causing an increase in serum amylase values cannot be elucidated from these cases. Future studies can focus on attempts to isolate *Bartonella* DNA from healthy and diseased pancreatic tissues in an attempt to confirm or disprove a possible association.

Ocular manifestations of *Bartonella* infections have been described previously in humans infected with *B. henselae* (9) and *B. grahamii* (15) and in cats infected with *B. henselae*. Chorioretinitis was noted only in dog 1; however, ocular lesions may not have been detected because a thorough ophthalmic examination was not performed in the other cases.

Bartonella spp. are capable of causing patent infections in several mammalian species, namely, human beings, cats, dogs and coyotes, cows, deer, rabbits, and rodents (4). The disease conditions associated with these organisms in nonreservoir hosts, such as humans and dogs, appear to be relatively similar, as evidenced by reports of endocarditis, peliosis hepatis, and granulomatous disease in canines and humans. This study adds another species (*B. elizabethae*) to the list of *Bartonella* organisms that can be detected in canine tissues and describes other potential disease manifestations that may be associated with *B. henselae* infection in dogs. As has been suggested previously (11), it may be necessary to apply molecular Koch's postulates to establish the pathogenicity of these highly adapted organisms that can persist within the vasculature for long periods of time.

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