Detection of *Bartonella henselae* DNA in two dogs with pyogranulomatous lymphadenitis

Sofia C. Morales, DVM; Edward B. Breitschwerdt, DVM, DACVIM; Robert J. Washabau, VMD, PhD, DACVIM; Ilze Matise, DVM, PhD, DACVP; Ricardo G. Maggi, PhD; Ashlee W. Duncan, MS

Case Description—1 dog evaluated because of inappetence and lameness of the left hind limb of 1 day's duration and 1 dog evaluated because of inappetence, fever, and lymphadenopathy of 2 weeks’ duration.

Clinical Findings—Histologic examination of excisional biopsy specimens from lymph nodes revealed pyogranulomatous lymphadenitis in both dogs. Quantitative real-time PCR assays detected *Bartonella henselae* DNA in blood samples and affected lymph node specimens from both dogs. Antibodies against *B henselae* were not detected via immunofluorescent antibody testing during active disease in either dog.

Treatment and Outcome—1 dog recovered after 6 weeks of treatment with doxycycline (5 mg/kg [2.3 mg/lb], PO, q 12 h), whereas the other dog recovered after receiving a combination of azithromycin (14.5 mg/kg [6.6 mg/lb], PO, q 24 h for 21 days), doxycycline (173 mg/kg [79 mg/lb], PO, q 24 h for 4 weeks), and immunosuppressive corticosteroid (prednisone [3 mg/kg [1.4 mg/lb], PO, q 24 h], tapered by decreasing the daily dose by 25% every 2 weeks) treatment.

Clinical Relevance—*B henselae* is implicated as a possible cause or a cofactor in the development of pyogranulomatous lymphadenitis in dogs. In dogs with pyogranulomatous lymphadenitis, immunofluorescent assays may not detect antibodies against *B henselae*. Molecular testing, including PCR assay of affected tissues, may provide an alternative diagnostic method for detection of *B henselae* DNA in pyogranulomatous lymph nodes. (*J Am Vet Med Assoc* 2007;230:681–685)

A 6-year-old neutered male Golden Retriever (dog 1) was evaluated by a veterinarian in Massachusetts for inappetence and lameness of the left hind limb of 1 day's duration. The dog had a history of atopy, food allergy, and hypothyroidism and was being treated with hydroxyzine (2.2 mg/kg [1 mg/lb], PO, q 12 h) and levothyroxine (20 µg/kg [9.1 µg/lb], PO, q 12 h). The dog had not received corticosteroid treatment during the year prior to evaluation. On physical examination, the dog had a poorly localized lameness of the left hind limb, bilateral conjunctivitis with prolapsed nictitating membrane, and bilateral mucopurulent ocular discharge. Cytologic examination of a sample obtained via conjunctival scraping revealed neutrophilic conjunctivitis. Results of a CBC were within reference limits. Mild hypoalbuminemia (2.4 g/dL; reference range, 2.7 to 3.7 g/dL) was the only abnormality detected on serum biochemical analyses. Analysis of urine obtained via cystocentesis revealed a specific gravity of 1.018 (reference range, 1.001 to 1.070), proteinuria (300 mg/dL; reference range, 0 to 1,000 mg/dL) through dipstick evaluation, and unremarkable sediment (3 to 10 RBC/hpf). The urine protein-to-creatinine ratio was 3.1 (values < 1 are considered normal), compatible with protein-losing nephropathy. No growth was detected on bacterial culture of urine. Borderline arterial hypertension (systolic pressure, 170 mm Hg; reference limit, < 150 mm Hg) was diagnosed by use of indirect blood pressure measurement. No abnormalities of the thorax, abdomen, pelvis, and both stifle joints were detected via radiography. Cytologic examination of samples obtained via arthrocentesis of multiple joints revealed neutrophilic arthritis consistent with an immune-mediated polyarthritis. Aerobic bacterial culture of joint fluid did not yield bacterial growth. Antibodies against *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Dirofilaria immitis*, *Ehrlichia canis*, and *Rickettsia rickettsii* were not detected via serologic testing. The dog was treated with carprofen (2.0 mg/kg [0.91 mg/lb], PO, q 12 h); doxycycline (5.4 mg/kg [2.5 mg/lb], PO, q 12 h); enalapril (0.54 mg/kg [0.25 mg/lb], PO, q 12 h); and an ophthalmic solution containing neomycin, polymyxin, and dexamethasone (1 drop in each eye, q 8 h).

Six days later, the dog was reevaluated for worsening of the conjunctivitis in the left eye. On physical examination, conjunctivitis in both eyes appeared worse.
with episcleritis of the left eye and superficial cervical and popliteal lymphadenopathy. Abdominal ultrasonography revealed moderate mesenteric lymphadenopathy. Histologic examination of an excisional biopsy specimen from the left popliteal lymph node revealed pyogranulomatous lymphadenitis (Figure 1). Staining of the biopsy specimen with Ziehl-Neelsen for acid-fast bacilli, Gomori methamine silver, and Warthin Starry silver stains did not reveal any organisms. Because of the potential for bartonellosis, IFA\(^1\) titers incorporating *Bartonella vinsonii* subsp *berkhoffii* and *Bartonella henselae* antigens and qPCR assays\(^2,3\) targeting DNA segments specific to *Bartonella* spp were performed on EDTA-anticoagulated blood and preparations of lymph node aspirates at the Vector Borne Diseases Diagnostic Laboratory at North Carolina State University. Bacterial culture for *Bartonella* spp was not requested. Antibodies against *B vinsonii* subsp *berkhoffii* and *B henselae* were not detected by use of IFA testing. Results of the qPCR assays that used DNA extracted from blood and lymph node samples were positive for *Bartonella* spp DNA by use of *Bartonella* genus probes and for *B henselae* by use of species-specific probes. By use of a conventional PCR assay\(^2,3\) a DNA amplicon of the appropriate size for *B henselae* was obtained from the lymph node aspirate. After cloning and DNA sequencing, analyses from the lymph node aspirate indicated that the 16S to 23S intergenic transcribed spacer region (GenBank accession No. AF369329) and the heme-binding phage-associated pap31 gene (GenBank accession No. AF308168) sequences were 100% homologous to *B henselae* strain San Antonio 2. The dog was treated with doxycycline (5 mg/kg [2.3 mg/lb], PO, q 12 h) for 6 weeks; however, the dog was lost to follow-up 2 weeks later. During the 8 weeks of follow-up, all clinical signs had resolved.

A 6-year-old neutered male English Springer Spaniel (dog 2) was referred to the University of Minnesota Veterinary Medical Center for evaluation of inappetence, fever, and lymphadenopathy of 2 weeks’ duration. Despite treatment with enrofloxacin (3 mg/kg, PO, q 24 h), metronidazole (15 mg/kg [6.8 mg/lb], PO, q 12 h), and carprofen (1 mg/kg [0.5 mg/lb], PO, q 12 h) for 7 days prior to evaluation, clinical signs persisted. On hospital day 1, the dog was febrile (rectal temperature, 40.7°C [105.2°F]) and had left mandibular lymphadenopathy. Mild thrombocytopenia (145,000 platelets/µL; reference range, 160,000 to 425,000 platelets/µL) was detected via CBC. Computed tomography identified left mandibular and right retropharyngeal lymphadenopathy. Excisional biopsies of both lymph nodes were performed on day 2. Pyogranulomatous lymphadenitis was diagnosed via histologic examination of biopsy specimens (Figure 2). Biopsy specimens were also stained with periodic acid-Schiff, Kinyoun stain for acid-fast bacilli, Gomori methamine silver, and Warthin Starry silver stains; no etiologic agents were detected. No growth was detected via fungal, aerobic, and anaerobic cultures of the lymph nodes. Serum antibodies against *Aspergillus* spp, * Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum* were not detected via agar gel immunodiffusion tests.\(^8\) Western blot analysis yielded a 3+ positive result (a dog with a value ≥ 3+ is interpreted by the laboratory as being infected), indicating serologic recog-

Figure 1—Photomicrograph of a section of a lymph node from a Golden Retriever evaluated for inappetence and lameness of the left hind limb of 1 day’s duration. Notice the presence of numerous macrophages admixed with few neutrophils and absence of normal lymphoid tissue. The histologic diagnosis was diffuse, marked, chronic pyogranulomatous lymphadenitis. H&E stain; bar = 50 µm.

Figure 2—Photomicrograph of a section of a lymph node from an English Springer Spaniel evaluated for inappetence, fever, and lymphadenopathy of 2 weeks’ duration. Notice discrete granuloma formation with epithelioid macrophages surrounding centrally located neutrophil aggregates. The histologic diagnosis was multifocal, marked, chronic pyogranulomatous lymphadenitis. Normal lymphoid tissue is not evident. H&E stain; bar = 50 µm.
Four months after surgery (day 130), the dog was reevaluated because of inappetence, fever, and generalized lymphadenopathy. Physical examination revealed mandibular, superficial cervical, inguinal, and popliteal lymphadenopathy. Fundic examination revealed bilateral chorioretinitis. Hematologic abnormalities included leukopenia (2,800 WBCs/µL; reference range, 4,100 to 13,300 WBCs/µL), neutropenia (1,340 neutrophils/µL; reference range, 2,100 to 11,200 neutrophils/µL) with band neutrophils (390 cells/µL; reference range, 0 to 130 cells/µL) with moderate toxic change, and thrombocytopenia (25,000 platelets/µL). Prothrombin time, activated partial thromboplastin time, and fibrin degradation products were within reference limits. No abnormalities were detected on radiographic views of the thorax; however, ventral displacement of the larynx detected on radiographic views of the head and cervical region suggested recurrent retropharyngeal lymphadenopathy. Cytologic examination of fine-needle aspirates obtained from the superficial cervical and inguinal lymph nodes revealed reactive lymphoid hyperplasia. Serum antibodies against A phagocytophilum, B burgdorferi, E canis, and R rickettsii were not detected via IFA testing. Further diagnostic evaluation and hospitalization were recommended but declined by the owner.

Bartonellosis was suspected because of the positive result obtained on western blot analysis on day 2 of hospitalization and the recurrent clinical signs without prior treatment for bartonellosis. Treatment with doxycycline (17.3 mg/kg [7.9 mg/lb], PO, q 24 h, for 4 weeks) and azithromycin (14.5 mg/kg [6.6 mg/lb], PO, q 24 h, for 21 days) was initiated. Three days later (day 133), the lymph nodes had subjectively decreased in size but the fever persisted. Radiography of the head and cervical region did not reveal evidence of retropharyngeal lymphadenopathy. Abnormalities detected via CBC included persistent leukopenia (3,100 WBCs/µL), thrombocytopenia (quantitation unavailable because of platelet clumping), and anemia (Hct, 38.0%; reference range, 38.5% to 56.7%). Although there was no definitive evidence of fungal infection, treatment with itraconazole (6 mg/kg [2.7 mg/lb], PO, q 24 h) was initiated. Nutritional support was given through a nasoesophageal tube. One week after reevaluation (day 140), the leukocyte (10,600 WBCs/µL) and neutrophil (7,210 neutrophils/µL) counts were within reference ranges; however, the left shift (420 band neutrophils/µL), anemia (Hct, 34.8%), and thrombocytopenia (32,000 platelets/µL) persisted. Serum biochemical abnormalities included hypoalbuminemia (1.9 g/dL; reference range, 2.7 to 3.7 g/dL), hyperglobulinemia (4.0 g/dL; reference range, 1.9 to 3.3 g/dL), hyperbilirubinemia (0.4 mg/dL; reference range, 0 to 0.3 mg/dL), hypoglycemia (73 mg/dL; reference range, 75 to 117 mg/dL), and high activities of alkaline phosphatase (316 U/L; reference range, 8 to 139 U/L) and aspartate transaminase (46 U/L; reference range, 16 to 44 U/L). Because of increased hepatic enzyme activity and continued inappetence, treatment with itraconazole was discontinued. Treatment with azithromycin and doxycycline and nutritional support via a nasoesophageal tube were continued. Antibodies were not detected in serum (blood collected on day 130) with an indirect IFA test performed at the Vector Borne Diseases Diagnostic Laboratory at North Carolina State University by use of B henselae and B vinsonii subsp berkhoffii antigens. Subsequently, qPCR amplification of Bartonella spp DNA was performed on 2 lymph node biopsy specimens that were acquired on day 2 and in serum from blood collected on day 140. After conventional PCR analysis of DNA from the lymph node, an amplicon of the appropriate size for B henselae was cloned and sequenced. The intergenic transcribed spacer region sequence was 99.7% (646/648 bases) homologous with B henselae (GenBank accession No. NC005956), and the pap31 gene sequence was 99.8% homologous with B henselae (GenBank accession No. AF308165), thus confirming infection with B henselae. Bartonella henselae DNA was also amplified from the blood sample.

Despite antimicrobial treatment, the dog’s clinical condition did not improve. Approximately 5 months after the initial evaluation, prednisone treatment was initiated at an immunosuppressive dosage (3 mg/kg [1.4 mg/lb], PO, q 24 h) because it was suspected that chronic bartonellosis had induced a secondary immunemediated component to the dog’s illness. Clinical signs completely resolved within 7 days. Physical examination on day 152 revealed resolution of the fever and lymphadenopathy. Findings on CBC included a stress leukogram (mature neutrophilia, 26,410 cells/µL), mild anemia (32.9%), and thrombocytosis (472,000 platelets/µL). Treatment with doxycycline and azithromycin was discontinued. Prednisone treatment was tapered by decreasing the daily dose by 25% every 2 weeks and eventually discontinued.

The dog was reevaluated 6 months after the initial evaluation. No abnormalities were detected via physical examination, CBC, and serum biochemical analyses. On day 244, 30 days after discontinuation of prednisone treatment, blood samples were submitted for Bartonella spp IFA titer and qPCR analysis; results of both were negative. Presently, the dog is clinically healthy and, according to its owners, has not had any signs of disease in 22 months.

**Discussion**

The genus Bartonella encompasses an expanding group of fastidious, aerobic, hemotropic, gram-negative, argyrophilic bacteria phylogenetically close to Rickettsia spp and other related alpha proteobacteria. Transmission of Bartonella spp among mammals is believed to be through the bite of an arthropod vector, with bites and scratches from mammalian reservoir hosts representing an alternative means of transmission. Presently, Bartonella spp have been associated with illness in dogs. Bartonella vinsonii subsp berkhoffii has been identified as an important cause of endocarditis and has been associated with cardiac arrhythmias, myocarditis, granulomatous rhinitis, anterior uveitis, and chorioretinitis. Bartonella clarridgeiae and Bartonella washoeensis, and Bartonella elizabethae have been associated with several clinical signs in dogs. Bartonella henselae has been implicated in peliosis hepatitis, granulomatous hepatitis, granulomatous sialoadenitis, and in 1 of the author’s (EBB) experience, endocarditis.
Bartonella quintana has been detected in 2 dogs with endocarditis.18

Bartonella henselae is the causative agent of CSD, a self-limiting, benign regional lymphadenopathy often accompanied by fever, malaise, and fatigue in immunocompetent humans.19 Atypical CSD, complicated by Paranaë'ïs ocuglandular syndrome, retinitis, glomerulonephritis, endocarditis, granulomatous hepatitis, hemolytic anemia, and osteomyelitis, develops in 5% to 25% of cases.19,20 Bartonella henselae causes several granulomatous syndromes in humans including granulomatous lymphadenitis, granulomatous hepatosplenitis, granulomatous osteitis, and orbital granulomas.21-24

Evidence of B henselae as a causative agent of granulomatous disease in dogs remains circumstantial. Bartonella henselae DNA was detected in liver biopsy specimens from a 4-year-old spayed female Basset Hound with granulomatous hepatitis, which even after antimicrobial treatment for bartonellosis, progressed to hepatic cirrhosis.13 Bartonella henselae DNA has been detected via PCR assay in a dog with granulomatous sialoadenitis.17

Molecular evidence suggesting B henselae as a possible cause or cofactor in the development of pyogranulomatous lymphadenitis in 2 dogs is provided by the present report. To the authors’ knowledge, this is the first report of identification of B henselae DNA in the lymph nodes of dogs. Detection of organisms in tissues by use of immunohistochemistry or in situ hybridization was not pursued because of the lack of assay availability and validity in dogs as well as lack of sensitivity in human patients with CSD.23,24

In dogs, pyogranulomatous inflammation can be elicited by injection site20 or foreign body reactions26; bacterial,27 fungal,27 and protozoal27 infections; or an aberrant immune response with no defined etiology.28 In the dogs reported here, etiologic agents were not detected by special histologic staining of affected lymph nodes, although specific molecular testing for other agents besides Bartonella spp was not performed. In dog 2, cultures from the affected lymph nodes did not reveal fungal or bacterial growth. Antibodies against infectious disease agents were not detected in either dog. The presence of B henselae DNA within affected lymph nodes and peripheral blood samples provides molecular evidence associating B henselae infection with pyogranulomatous lymphadenitis in these 2 dogs. The complete response to doxycycline in dog 1 and the partial response to doxycycline in dog 2 suggested a bacterial etiology in both cases. The lack of underlying etiologic agents, the molecular evidence of B henselae DNA in affected tissues, and the response to antimicrobial treatment make the association between B henselae and pyogranulomatous lymphadenopathy in dogs plausible, although coinfection with other, yet unidentified, agents is possible. The need for immunosuppression for complete clinical remission in dog 2 suggested an immune-mediated component to the dog’s illness, which could have been caused by an underlying B henselae infection.

Interestingly, after antimicrobial treatment in dog 1 and antimicrobial and immunosuppressive corticosteroid treatment in dog 2, there was resolution of the dogs’ accompanying clinical signs. Whether there is an absolute need for antimicrobial or immunosuppressive treatment in dogs with bartonellosis is not known. The use of antimicrobials for classical CSD in humans is controversial because antimicrobial administration does not appear to modify the clinical course of CSD.29 Although antimicrobials should be the logical mainstay of treatment for bartonellosis in dogs, concurrent administration of anti-inflammatory or immunosuppressive drugs may be necessary to modulate an aberrant immune response that has developed in conjunction with chronic infection.

The 2 dogs of this report did not have detectable antibodies against B henselae during active disease, as determined by use of IFA testing. Potential explanations for this finding include low antibody concentrations attributable to previous antimicrobial treatment, lack of sensitivity of the IFA assay, and immunogenic anergy from primary host immunosuppression by B henselae or by sequestration of Bartonella spp antigen in intracellular compartments. Immunofluorescent antibody titers4 are highly variable and decline after experimental inoculation of B vinsonii subsp berkhoffii in dogs.30 Results of a study31 in cats indicate a relapsing bacteremia with a highly variable humoral immune response among individual cats experimentally infected with B henselae. The sensitivity and specificity of IFA titers for antibodies against B henselae in humans suspected of having CSD ranges from 14% to 100% and 34% to 100%, respectively, depending on the assay used (depending on factors such as the antigen used for the test) and on the definitions of cases and controls used to establish sensitivity and specificity, in addition to cutoff values.32,33 The sensitivity and specificity of IFA and PCR testing in dogs infected with B henselae are not known. The potential lack of IFA test sensitivity for detection of antibodies against B henselae in dogs with pyogranulomatous lymphadenitis emphasizes the need for prospective studies that incorporate use of bacterial culture, serologic testing, and PCR assays. These studies will help to determine the clinical use of acute and convalescent antibody titers for detection of seroconversion or low-level antibody production in dogs infected with B henselae.

The pathogenicity of B henselae in dogs has not been clearly established. Presently, B henselae should be considered as a potential cause or cofactor for pyogranulomatous lymphadenitis in dogs. In dogs with pyogranulomatous lymphadenitis, serologic testing may not detect antibodies against B henselae. Molecular testing, including PCR assay of affected tissues, provides an alternative diagnostic method for detection of B henselae DNA within pyogranulomatous lymph nodes.
References


