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CASE REPORT

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Suspected *Bartonella* osteomyelitis in a dog

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Abstract

Bartonella associated osteomyelitis, while described in humans and a cat, has to our knowledge not been described in dogs. Infection with *Bartonella* spp. should be considered as a potential bacterial cause of osteomyelitis in dogs.

KEYWORDS

bone, infection, kidney, polydipsia and polyuria

1 | INTRODUCTION

Bartonella spp. are fastidious, hemotropic, gram-negative bacteria that are transmitted by arthropod vectors, blood transfusion, needle sticks, or via animal scratches or bites.[1] Dogs and humans tend to develop very similar pathologies when infected with a *Bartonella* spp.[2] In humans, bartonellosis has been associated with a wide range of symptoms and organ system pathology, including cat-scratch disease (CSD), bacillary angiomatosis, hepatic/splenic peliosis, endocarditis, granulomatous hepatitis, panniculitis, osteomyelitis, and neurobartonellosis.[3] While considered a rare phenomenon, osteomyelitis in CSD patients, secondary to *Bartonella henselae* (*Bh*), is well described in the human medical literature.[4,5] Bone pain and fever often occur in association with a prolonged course of illness. In greater than 70% of reported CSD patients, bone involvement is limited to one anatomic site. Importantly, primary osteomyelitis often occurs without other historical or systemic manifestations of CSD. In contrast to the human literature, we are not aware of previous reports of Bartonella osteomyelitis in dogs.

In dogs, *Bartonella* infection has been associated with varying clinical abnormalities, including splenomegaly, nasal discharge, epistaxis, and lameness.[6] Pathological associations have included localized or generalized inflammatory diseases such as uveitis, endocarditis, myocarditis, panniculitis, polyarthritis, lymphadenitis, various nervous system disorders including transverse myelopathy, neutrophilic or granulomatous meningoencephalitis and meningitis, splenic lymphoid hyperplasia, and hemangiosarcoma.[1,7] To date, *Bartonella vinsonii* subsp. *berkhoffii* (Bvb) osteomyelitis in a cat represents the only published case of osteolytic and osteoproliferative lesions involving a companion animal.[8]

To report the diagnostic evaluation, treatments, and follow-up of *Bartonella*-associated osteomyelitis in a dog. We report an 8-year-old male-castrated American Bulldog was examined due to a history of polyuria and polydipsia and left cubital joint effusion accompanied by lameness. Radiographically, there was osteolysis and osteoproliferation of the left humeral medial epicondyle. Serology documented elevated *Bartonella henselae*, *Bartonella koehlerae*, *and Bartonella vinsonii* subsp. *berkhoffii* indirect immunofluorescent antibody (IFA) titers. Doxycycline,

enrofloxacin, and clarithromycin were administered sequentially and in combination with treatment of Bartonella osteomyelitis, while monitoring seroreversion. In conjunction with extended antibiotic administration, there was improvement in clinical, hematologic, and radiographic abnormalities. Seroreversion, based upon convalescent *Bartonella* spp. antibody titers, supported a diagnosis of Bartonella osteomyelitis. Infection with *Bartonella* spp. appears to represent a previously unrecognized cause of osteomyelitis in dogs. Seroreversion in conjunction with improvement and resolution of all clinical and hematological abnormalities supported therapeutic elimination of pathogenic *Bartonella* spp.

Clinical significance

When considering *Bartonella* spp. bone infection, serology, extended culture in specialized growth medium, and collection of tissue for PCR testing should be considerations to achieve a definitive diagnosis.

2 | CLINICAL REPORT

On January 12, 2017, a 48 kg, 8-year-old male-castrated American Bulldog was examined at Fort Hunt Animal Hospital for a 10-day history of polyuria/polydipsia (PU/PD). Rectal temperature was normal (38°C). A complete blood count (CBC) was unremarkable. Hyperglobulinemia (4.3 g/dl; reference range, 2.4–4.0) was the only serum chemistry abnormality. Urine specific gravity was 1.006 with negative urine dipstick indicators and an inactive urine sediment examination. One week later, the dog was examined for intermittent limping on the left forelimb. Severe pain was elicited on manipulation of the left cubital joint. Radiographic abnormalities were not visualized in left cubital joint. Abdominal ultrasonography identified small hypoechoic nodules in the liver and spleen. A rapid assay test (SNAP 4DxPlus, IDEXX Laboratories, Inc) for *Borrelia burgdorferi*, *Ehrlichia*, *Anaplasma* spp. antibodies and *Dirofilaria immitis* antigens was negative. The dog was discharged with instructions to administer gabapentin and tramadol for pain control, with a tentative plan to schedule splenectomy and obtain liver biopsies. Five days later, the dog acutely developed non-ambulatory paraparesis involving the hind limbs, suggestive of transverse myelitis. Structural abnormalities were not visualized on spinal radiographs. As multiple myeloma was a differential diagnostic consideration, serum protein electrophoresis confirmed a polyclonal, rather than monoclonal gammopathy, indicative of chronic antigenic immune stimulation.

Instead of splenectomy, ultrasound-guided fine needle splenic aspirates identified lymphoid hyperplasia. On 1/25/17, the dog was referred for additional diagnostic evaluation. Physical examination abnormalities included bilateral cubital joint pain and effusion. Elbow arthrocentesis documented neutrophilic inflammation in both cubital joints. There were occasional large basophilic round cells in the left elbow joint, indicative of reactive synovial cells or potentially neoplastic synoviocytes. Sera submitted to the NCSU-VBDDL documented a *Bh* indirect immunofluorescent antibody (IFA) titer of 1:64. *Bartonella koehlerae* and *Bvb* IFA titers were negative (<1:16). Doxycycline 450 mg (9.375 mg/kg) q24h and prednisone 40 mg (0.83 mg/kg) q24h were dispensed for 6 weeks. The dog improved clinically, including resolution of the PU/PD; however, elbow swelling and left foreleg lameness persisted.

On March 6th, the dog experienced a relapse of non-ambulatory paraparesis involving the hindlimbs. The *Bh* IFA titer, 6 weeks after the initial titer and after 6 weeks of doxycycline/prednisone administration, was again 1:64 (University of California, Davis Microbiology Department). Physical examination confirmed persistent left cubital swelling, more severe medially. Compared to prior radiographs, there was now osteolysis and irregular osteoproliferation of the medial epicondyle of the left humerus (Figure 1). The radiographic changes were consistent with osteomyelitis of either bacterial or fungal etiology. Based upon the historical progression, other causes of aggressive bone lesions (ie, primary or metastatic osseous neoplasia) were considered less likely. Repeat arthrocentesis of the left cubital joint documented only mesenchymal cell proliferation. Following a phone consultation (3/24/17) with an internist, enrofloxacin 476 mg (10 mg/kg) q24h was added to the doxycycline regimen for treating presumptive Bartonella osteomyelitis and the prednisone dose was tapered.

Beginning in January 2017, the dog was treated for presumptive Bartonella osteomyelitis for a total of 7 months with doxycycline 450 mg (9.375 mg/kg) q24h and 4 months of concurrent enrofloxacin 476 mg (10 mg/kg) q24h. During this treatment period, the owner reported a progressive increase in energy level, but the dog remained intermittently lame with persistent swelling of the distal left forelimb. In July 2017, a CBC and serum biochemistry panel were unremarkable; other than a mildly elevated SDMA (15ug/dl; reference range, 0–14). Serum globulins had normalized (3.8 g/dl, reference range, 2.4–4.0). Urine specific gravity was concentrated at 1.052, with urine dipstick and sediment examinations within normal reference ranges. Repeat NCSU-VBDDL serology documented titers of 1:32, 1:256, and 1:256 to *Bh*, *Bvb*, and *Bk*, respectively. Based upon a treatment regimen used in a previous published case report, and at the recommendation of the consulting internist, clarithromycin 250 mg (5 mg/kg) q12h was added to the antibiotic regimen of doxycycline and enrofloxacin.[9]

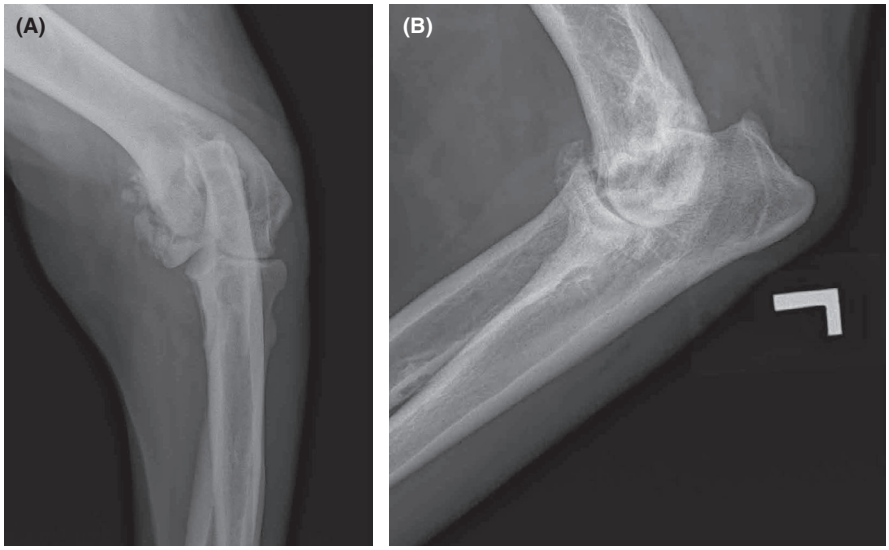

FIGURE 1 Radiographs of the left cubital joint. (A) Craniocaudal and (B) lateral radiographic projections of the left cubital joint with evidence of osteolysis and irregular osteoproliferation of the medial epicondyle of the left humerus with moderate soft tissue swelling

When re-evaluated by the attending veterinarian on September 13th, ambulation and joint effusion were greatly improved. Physical examination indicated a mild left forelimb lameness. A CBC was unremarkable. Mild hyperlipasemia was the only serum biochemical abnormality (974 U/L; reference range, 138–755). Urine specific gravity was 1.033, with negative urine dipstick and urine sediment examinations. SDMA was normal (12 μg/dl; reference range, 0–14). NCSU-VBDDL IFA B*artonella* titers were 1:64, <1:16, and <1:16 to *Bh*, *Bvb*, and *Bk*, respectively. Enrofloxacin, doxycycline, and clarithromycin (at previously recommended doses) were continued until October 21st when a recheck examination documented negative IFA antibody titers (<1:16) to all three *Bartonella* spp. Radiographically, there was substantial improvement of the osteolysis and proliferation of the medial epicondyle of the left humerus (Figure 2).

During a routine wellness examination on February 26, 2018, the owner reported only mild intermittent left forelimb lameness. As of August 2020, the dog remained well with no significant lameness or other systemic signs of illness.

3 | DISCUSSION

We describe a dog with left forelimb lameness, two bouts of transient posterior paresis, polyuria, polydipsia, hyposthenuria, hyperglobulinemia, and hepatic and splenic ultrasonographic abnormalities that developed osteomyelitis in association with serological evidence of *Bartonella* infection; *Bh* seroreactivity was documented at sequential illness time points at two academic diagnostic laboratories. Based upon serology, the dog was most likely infected with *B. henselae*; however, co-infection with more than one *Bartonella* spp., as previously reported in dogs, was possible.[10] Clinical (lameness and polydipsia/polyuria), serum biochemical (hypergammaglobulinemia), and radiographic improvement occurred in conjunction with a protracted course of antibiotics and seroreversion.

Polyarthritis associated with *Bartonella* infection has been reported in dogs.[6,11,12] In dog's *Bh* seroreactive, neutrophilic polyarthritis was confirmed via cytology following arthrocentesis.[11] In another study, *Bartonella* spp. seroreactivity was significantly associated with lameness and arthritis in dogs, including polyarthritis, when compared to non-seroreactive control dogs.[12] Using an enrichment culture approach, *Bh* and *Bvb* were isolated from a dog's synovial fluid and from the synovium and excised femoral head from a veterinarian with rapidly progressive osteoarthritis.[10,13] Interestingly, the *Bh* and *Bvb* co-infected dog progressed from a non-erosive to erosive arthritis. Treatment with doxycycline as a sole antibiotic did not result in elimination of either bacteria from the stifle joints.[10] In retrospect, that dog would have potentially benefited from combination antibiotic therapy, as used in this case. It is possible that administration of nearly 1 mg/kg of prednisone in conjunction with doxycycline during the initial 6 weeks of therapy compromised the bacteriostatic response to doxycycline in this osteomyelitis case.

As previously proposed, isolating the same infectious agent(s) from a naturally occurring pathological entity found in at least three different mammalian genera may assist clinicians in the establishment of disease causation.[2] Bartonella osteomyelitis was previously described in a cat[8] and in humans.[13,14] As *Bartonella* spp. have been implicated in association with osteomyelitis in cats, this dog and humans, this lesion now satisfies the postulate for comparative infectious disease causation. Osteomyelitis associated with *Bartonella* infection was first described in human patients in 1954 and is considered one of several CSD-associated musculoskeletal manifestations caused by *B. henselae*.[15] While considered a rare CSD presentation, human case reports referencing this association have increased with the advent of serological and PCR testing, particularly in pediatric patients with

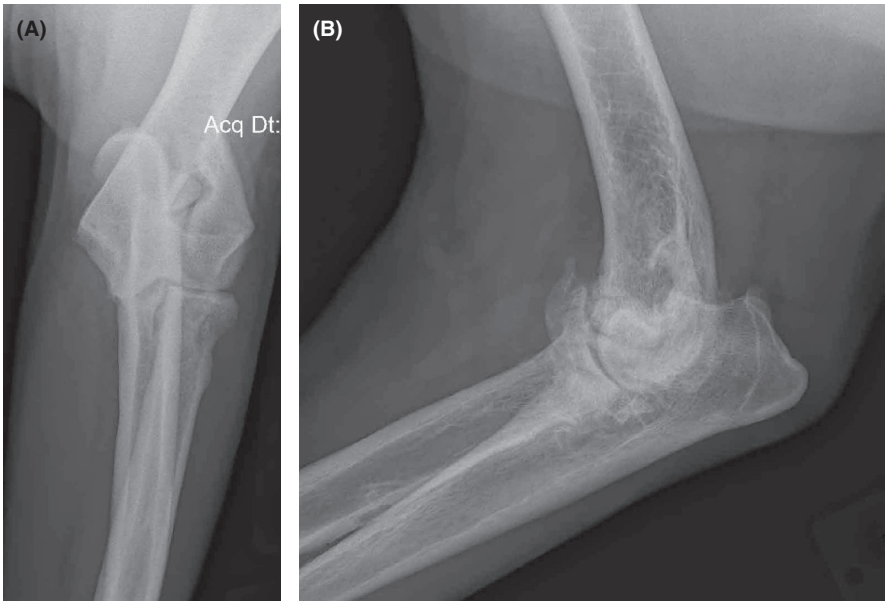

FIGURE 2 Radiographs of the left cubital joint. (A) Craniocaudal and (B) lateral radiographic projections of the left cubital joint with improvement in the previously identified osteolysis and irregular osteoproliferation of the medial epicondyle of the left humerus and associated soft tissue swelling

osteomyelitis, often involving vertebral sites.[4,15] In one study, chronic musculoskeletal complications including myalgia, arthritis, and osteomyelitis were reported in 10% of CSD cases.[16] Radiographic abnormalities consist of lytic lesions, often with sclerosis or periosteal reaction surrounded by soft tissue swelling.[4,5] Osseous involvement in CSD can be multifocal, but most often only a single osteolytic lesion is reported.[4]

Bacterial isolation of Bartonella is challenging because of fastidious nutritional growth requirements, intracellular tropism, and a relapsing bacteremia.[17] Limitations to bacterial isolation include the need for sampling prior to antibiotic therapy, specialized growth requirements, a prolonged incubation period, and the need for enrichment culture.[18] Nucleic acid amplification following enrichment culture using *Bartonella* alpha-Proteobacteria growth medium (BAPGM) and the recent addition of ddPCR to the enrichment platform has improved the microbiological detection of *Bartonella* spp.[19-21] Unfortunately, as bartonellosis was not an initial diagnostic consideration, blood and joint fluid samples were not obtained for BAPGM enrichment blood, joint fluid, or bone biopsy culture, as performed for the veterinarian with rapidly progressive osteoarthritis.[13] In dogs, *Bartonella* IFA serology is highly specific (97% specificity for each of the three IFA antigens used in this study) and remains the current diagnostic "gold standard".[22] Unfortunately, IFA sensitivity is poor, as previous studies in dogs have documented a lack of seroreactivity, despite documentation of bacteremia by isolation or PCR.[7,17,23] Further complicating the diagnosis of bartonellosis is the tendency of the bacteria to form localized infections and the development of biofilms.[24,25]

Current recommendations for the treatment of *Bartonella* musculoskeletal infections are based predominantly on empirical data. In humans with *Bh* osteomyelitis, prognosis is good with patients being treated for a median duration of 32 days with mono, dual, or triple antibiotic therapy.[5] As an optimal treatment regimen for canine bartonellosis has not been established, the antibiotic combinations in this dog were selected on the basis of published in vitro sensitivity data and evolving data relative to antibiotic treatment outcomes.[9,26,27]

Persistent *Bvb* infection was documented in the cat with osteolytic and osteoproliferative lesions, as pathogen DNA was amplified 15 months apart.[8] Sequential serology appears to be useful to support therapeutic elimination of Bartonella infections in dogs.[6,9] Initial testing by two laboratories documented only *Bh* seroreactivity. Importantly, experimental infection of dogs with different *Bartonella* spp. resulted in a species and genotype-specific serological response during the 3-months post-infection.[28] Whether the July 2017 serology results represented historical co-infection, recent infection with *Bvb* and *Bk*, or recognition of common antigenic epitopes induced by microbial injury secondary to antibiotics could not be determined. *Bartonella* spp. co-infections are not uncommon in dogs.[17]

Interestingly, the dog in this report was initially examined due to a recent onset of PU/PD. Hyposthenuria was documented in conjunction with normal renal function. Sequential urinalyses failed to implicate infection, inflammation, or proteinuria. Hyposthenuria is associated with central diabetes insipidus, renal diabetes insipidus, and psychogenic polydipsia with secondary polyuria. In this dog, concentrated urine specific gravities and resolution of the PU/PD occurred in association with antibiotic administration. Pathophysiologically, *Escherichia coli* endotoxin has been implicated as a cause of hyposthenuria in dogs.[29,30] Recently, we describe children infected with *Bartonella* spp. who experienced intense thirst.[31] Examination by a nephrologist documented low urine specific gravity

measurements, but failed to confirm a cause for the abnormally increased water intake. Whether infection with Bartonella contributes to increased thirst or alterations in urine concentrating ability awaits future studies.

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We appreciate the cooperation of the dog's owner in facilitating follow-up examinations and approving the publication of this case report.

CONFLICT OF INTEREST

In conjunction with Dr. Sushama Sontakke and North Carolina State University, Edward B. Breitschwerdt, DVM holds U.S. Patent No. 7115385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is a co-founder, shareholder and Chief Scientific Officer for Galaxy Diagnostics, a company that provides advanced diagnostic testing for the detection of *Bartonella* species infections. All other authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

TT: provided primary care for the dog in this case report. FE: generated the draft manuscript. EB: provided consultation, encouraged the publication of the case, and contributed to the content and discussion of the case in the final manuscript submission.

ETHICAL APPROVAL

The owner of the dog in this case report was fully supportive of and provided permission for the publication of our findings.

CONSENT STATEMENT

Published with written consent of the patient.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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