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Bartonella rochalimae, *a* newly recognized pathogen in dogs

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Abstract

Background: *Bartonella* spp. comprise a genus of bacteria that frequently cause persistent, often subclinical infection. Although many *Bartonella* spp. have been implicated in a variety of clinical presentations, *Bartonella rochalimae* has yet to be documented in association with a clinical presentation other than infectious endocarditis (IE) in dogs.

Objectives: To document a spectrum of clinical presentations accompanied by mild hematological abnormalities in *B rochalimae*-infected dogs from the United States.

Animals: Eight dogs with documented *B rochalimae* infection.

Methods: Retrospective 10-year study of *B rochalimae* naturally infected dogs. Clinical and clinicopathologic data, including medical history, CBC, serum biochemistry panel, urinalysis, echocardiogram, and comprehensive vector-borne disease diagnostic panel results, were reviewed.

Results: Eight dogs were diagnosed with *B rochalimae* via polymerase chain reaction (PCR) amplification. Five dogs were diagnosed with IE. Three dogs, PCR positive for *B rochalimae*, were diagnosed with seizures or antibiotic responsive lameness or during routine screening of a military working dog.

Conclusions: This case series provides support for an association between *B rochalimae* and IE and provides documentation of dogs infected with *B rochalimae* with other clinical diagnoses.

KEYWORDS

emerging, endocarditis, infection, polymerase chain reaction, vector-borne

1 | INTRODUCTION

Bartonella spp. comprise a genus of fastidious, vector-borne, gram-negative bacteria that frequently cause persistent and often subclinical intraerythrocytic and endotheliotropic infection in highly adapted reservoir hosts.[1-4] The mutualistic relationship between mammalian hosts, arthropod vectors, and *Bartonella* spp. has a long-standing evolutionary basis, as exemplified by *Bartonella henselae* coevolution with fleas (*Ctenocephalides felis*) and domestic cats.[1-4] For instance, *B henselae* DNA has been amplified from the dental pulp of 800-year-old French cats.[5] Strikingly, asymptomatic *Bartonella* spp. bacteremia in reservoir hosts, such as bats, feral cats, and rodents, have been reported in a large proportion of the respective study populations.[6-8] Although the clinical implications of persistent infection are not fully understood, transmission of a reservoir-adapted *Bartonella* spp. to an accidental host, such as a dog, appears to more likely result in the eventual development of disease manifestations, potentially months to years after transmission. Because of the fastidious microbiological

Abbreviations: CSF, cerebrospinal fluid; CVBPs, canine vector-borne pathogens; CVM, College of Veterinary Medicine; ELISA, enzyme-linked immunosorbent assay; HCT, hematocrit; IE, infectious endocarditis; IFA, immunofluorescent antibody; ITS, intergenic transcribed spacer; MST, median survival time; NCSU, North Carolina State University; PCR, polymerase chain reaction; PDA, patent ductus arteriosus; qPCR, quantitative polymerase chain reaction; VBDDL, Vector Borne Disease Diagnostic Laboratory; WBC, white blood cell.

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nature of these organisms, the often ill-defined clinical signs associated with infection, and the relatively small volume of currently available clinical literature, the relevance of bartonellosis as a cause of chronic illness in dogs is yet to be clarified.[3,9-11]

Despite current evidence-based medicine limitations, a substantial number of recent publications associate *Bartonella* spp. with chronic illnesses in both animals and humans.[12-20] While *Bartonella* spp. have been associated with a variety of clinical disease manifestations, the most convincing evidence for causation exists between *Bartonella* spp. and infectious endocarditis (IE); an association that is being made with increasing frequency in cats, dogs, and humans.[20] *Bartonella* spp. implicated in association with IE in dogs include *Bartonella quintana*, *Bartonella clarridgeiae*, *B henselae*, *Bartonella koehlerae*, *Bartonella washoensis*, *Bartonella vinsonii* subsp. *berkhoffii* genotypes I, II, III, and IV, and most recently, *Bartonella rochalimae*.[14-16,18,21] *Bartonella rochalimae* IE was first reported in 2008 in a dog from California.[22] When DNA amplified from the dog's aortic valve (obtained on postmortem examination) was compared with previously deposited GenBank *Bartonella* DNA sequences, the best match was with DNA sequences obtained from a woman with a travel history to Peru, who was infected with *B rochalimae*.[22] In a recent publication from Spain, medical records of 30 dogs with blood culture negative endocarditis were retrospectively reviewed.[16] In the Spanish study, *B rochalimae* was overrepresented (6 of 8 *Bartonella* polymerase chain reaction [PCR] positive endocarditis cases) in dogs with blood culture negative endocarditis.[16] Heart valves of the 2 remaining dogs were infected with *B vinsonii* subsp. *berkhoffii* and *B koehlerae*, respectively.

While the association with IE is particularly strong, *Bartonella* spp. have also been identified in dogs with fever,[23] uveitis,[24] lymphadenopathy,[25] arthritis,[26] vasculitis,[27] and signs of neurologic disease.[28] Because of the fastidious nature of *Bartonella* spp. (22-24 hours dividing time, similar to *Mycobacteria*),[29] and the propensity to cause chronic, subclinical bacteremia, the potential causative link between disease manifestations other than endocarditis warrants further exploration. The purpose of our study was to review the clinicopathologic findings in dogs infected with *B rochalimae*, based upon antemortem PCR amplification and DNA sequence confirmation of the infecting *Bartonella* sp.

2 | ANIMALS, MATERIALS, AND METHODS

2.1 | Medical records

A database containing canine vector-borne pathogen (CVBP) diagnostic testing results generated at the NCSU-CVM-VBDDL between 2010 and 2019 was reviewed for dogs with PCR-confirmed *B rochalimae* infection. Dogs were eligible for study inclusion if the CVBP-PCR panel was positive for *B rochalimae* and medical data were concurrently obtainable for review. To expand on the data available for analysis, PCR and serology were performed on stored samples (blood/DNA/serum), when available, for cases that were PCR+ for *B rochalimae* but did not have complete CVBP testing performed at the time of initial sample submission.

Eight dogs were identified for inclusion based on PCR amplification of *B rochalimae* at the NCSU-CVM-VBDDL and their medical records were retrospectively reviewed. Case data included breed, age, sex, presenting complaint, CBC, serum chemistry, and clinical diagnosis. Dogs were not excluded based on seroreactivity to other CVBPs.

Diagnostic testing included immunofluorescent antibody (IFA) assays for *Babesia canis*, *Babesia gibsoni*, *B henselae*, *B koehlerae*, *B vinsonii* subsp. *berkhoffii*, *Ehrlichia canis*, and *Rickettsia rickettsii*; whole blood PCR assays for *Anaplasma* spp., *Bartonella* spp., *Babesia* spp., *Ehrlichia* spp., hemotropic *Mycoplasma* spp., and *Rickettsia* spp.; and a commercial enzyme-linked immunosorbent assay (ELISA) (SNAP 4DX Plus, IDEXX Laboratories, Inc, Westbrook, Maine) for *Anaplasma* spp. (*A phagocytophilum* and *A platys*), *Borrelia burgdorferi*, and *Ehrlichia* spp. (*E canis*, *E chaffeensis*, and *E ewingii*) antibodies, and *Dirofilaria immitis* antigen. All serum, whole blood, and tissue samples were tested by the NCSU-VBDDL (Raleigh, North Carolina). Seroreactive samples were defined as having end-point IFA titers ≥1 : 64.

2.2 | Retrospective testing of PCR primers

Recently, Chan et al described a sensitive and specific PCR platform for amplification of *B rochalimae* DNA, by targeting three, rather than one, *B rochalimae* genes: *gltA*, *rpoB*, and the intergenic transcribed spacer (ITS) region.[30] Historically, our laboratory has targeted the *Bartonella* 16S-23S ITS region using different primer combinations.[31] Kosoy and colleagues described a genus-specific real-time PCR assay targeting the *ssrA* gene, for detection and differentiation of *Bartonella* spp. and genotypes.[32] To examine the relative amplification efficiency of the 3 primer sets used in our laboratory, DNA was extracted from 5 stored, residual EDTA-anti-coagulated blood samples. Primer combinations were ITS 325s-1100as and ITS 425s-1100as as previously described,[31] and modified *ssrA* gene primers,[32] as described in our study. Residual stored blood was not available from 2 dogs for DNA extraction and PCR testing with the 3 primer sets.

2.3 | DNA extraction and ssrA qPCR

Extractions were performed using a QIAsymphony SP robot (QIAGEN, Valencia, California) and QIAsymphony DNA Mini Kit (QIAGEN; catalog no. 931236) or a Qiagen BioRobot M48 Robotic Workstation with MagAttract DNA Mini M48 kit (QIAGEN; catalog no. 953336) depending on the time of sample submission. The absence of PCR inhibitors was demonstrated by the amplification of glyceraldehyde-3-phosphate dehydrogenase. Primers for *ssrA* quantitative polymerase chain reaction (qPCR) assays included: ssrA-F (5′ GCT ATG GTA ATA AAT GGA CAA TGA AAT AA 3′) and ssrA-R3 (5′ GAC GTG CTT CCG CAT AGT TGT C 3′) to amplify an approximate 208 base pair region of *ssrA*. Amplification reactions contained 12.5 μL SsoAdvanced Universal SYBRGreen Supermix (Bio-Rad, Hercules, California), 5 μL DNA template, primers at 0.4 μM final concentration, and molecular grade water to a final volume of 25 μL. Thermocycler conditions consisted of an initial denaturation step at 98°C

for 3 minutes, followed by 40 cycles at 98°C for 15 seconds, 62°C for 15 seconds, and 72°C for 15 seconds. Melting temperature (T_m) measurements were made between 65°C and 90°C at 0.5-second intervals, where positive *Bartonella* spp. melting temperatures ranged from 80°C to 82.5°C. All qPCRs included a positive control consisting of *B henselae* ssrA plasmid DNA and negative controls, including a no-template control consisting of filter-sterilized, molecular-grade water and uninfected canine genomic DNA. Sequencing of amplicons was performed by GENEWIZ Inc (Research Triangle Park, North Carolina) and alignments made with GenBank reference sequences using the AlignX software (Vector NTI Suite 6.0, Invitrogen).

3 | RESULTS

Bartonella rochalimae DNA was PCR-amplified and sequenced from blood or tissue specimens for the 8 dogs included in our study. Platelet count, hematocrit (HCT), and white blood cell (WBC) counts were available for all dogs at the time of initial presentation for the clinical complaint that led to CVBP testing. Reference intervals varied based on the diagnostic laboratory to which the samples were submitted. Based on laboratory reference intervals, 4 of 8 dogs were anemic, and 4 of 8 dogs were thrombocytopenic. Four dogs had a leukocytosis, all characterized by a mature neutrophilia without a left shift and 1 dog an eosinophilia. A brief medical history is provided for each dog.

3.1 | Dog 1

A 3-year-old-male intact German Shepherd Dog from Virginia first exhibited malaise in May 2018. When progressive weight loss, a new onset heart murmur, and azotemia were documented in June 2018, diagnostic testing included a CBC, abdominal ultrasound, echocardiogram, and vector borne disease serology/PCR testing. Hematological abnormalities included thrombocytopenia (platelet count of 75 000/μL) and leukocytosis (WBC count 17 900/μL). A mild hyperglobulinemia was identified on serum biochemistry with globulins 5.3 g/dL (upper reference interval 5.2 g/dL). Aerobic urine culture did not grow bacteria. The dog was IFA seroreactive to *B henselae* (1 : 8192), *B vinsonii* subsp. *berkhoffii* (1 : 2048), and *B koehlerae* (1 : 8192) and *R rickettsii* (1 : 128) antigens. *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, and *Rickettsia* PCR assays were negative. Echocardiographic abnormalities included aortic valve vegetation with secondary severe aortic valve stenosis, mitral regurgitation, and left ventricular concentric and eccentric hypertrophy. *Bartonella rochalimae* DNA was amplified from the dog's blood, supporting a diagnosis of IE. Treatment included doxycycline (~6.6 mg/kg PO in the morning and 3.3 mg/kg in the evening; Vibramycin, Pfizer, New York, New York) and enrofloxacin (~5.7 mg/kg PO once daily; Baytril, Bayer, Whippany, New Jersey); the dog is still receiving these antibiotics 21 months later, at the time of manuscript submission. Because of the initial IE diagnosis, the dog has experienced 3 episodes of left-sided congestive heart failure (CHF). Current medical treatment for CHF includes furosemide (Furosemide, Pfizer), spironolactone (Aldactone, Pfizer), and enalapril (Vasotec, Valeant Pharmaceuticals North America, Bridgewater, New Jersey). Clopidogrel (Plavix, Bristol-Myers Squibb, New York, New York) is being administered to prevent thromboembolism. Overall, the dog continues to experience a good quality of life.

3.2 | Dog 2

A 3-year-old-female spayed hound from North Carolina was diagnosed with a patent ductus arteriosus (PDA) and pulmonic stenosis. In March 2018, the dog had a cough and was radiographically diagnosed with left-sided CHF. She responded well to medical treatment for CHF and was referred for possible PDA occlusion. On presentation to the referral center, platelet count was within the reference range (200 000/μL). There was a mild leukocytosis (WBC count 14 340/μL) and a mild hyperglobulinemia at 4.1 g/dL (upper reference interval 3.8 g/dL). Aerobic urine culture did not result in bacterial growth. SNAP 4DX ELISA was negative. The dog was IFA seroreactive to *B henselae* (1 : 2048), *B vinsonii* subsp. *berkhoffii* (1 : 256), and *B koehlerae* (1 : 1024) antigens. *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, *and Rickettsia* PCR assays were negative. *Bartonella rochalimae* DNA was PCR-amplified and sequenced from the dog's blood. Echocardiographic abnormalities included severe eccentric left ventricular hypertrophy, a moderately dilated left atrium, mild valvular pulmonic stenosis, severe pulmonary regurgitation, a mildly dilated pulmonary artery, PDA, and a hyperechoic (approximately 0.7 × 0.3 cm) independently oscillating lesion on the left cusp of the pulmonic valve. *Bartonella rochalimae* vegetative endocarditis of the pulmonic valve was diagnosed. Treatment was initiated with doxycycline (~5.3 mg/kg PO twice daily; Vibramycin, Pfizer) and enrofloxacin (9.7 mg/kg PO once daily; Baytril, Bayer) with the recommendation to repeat echocardiogram after 6 weeks of treatment and consider surgical closure of the PDA. The dog was lost to follow-up.

3.3 | Dog 3

A 2-year-old-female spayed Beagle from North Carolina was examined because of hematochezia, fever, and hind limb lameness. The dog was thrombocytopenic (platelet count 39 000/μL). The leukocyte count was 16 650/μL (upper reference interval 16 760/μL). Serum globulins were within the reference interval at 2.9 g/dL. SNAP 4DX ELISA was negative. The dog was IFA seroreactive to *B henselae* (1 : 128), *B vinsonii* subsp. *berkhoffii* (1 : 128), *B koehlerae* (1 : 512), and *Babesia canis* (1 : 128) antigens. *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, and *Rickettsia* PCR assays were negative. *Bartonella rochalimae* DNA was PCR amplified and sequenced from the dog's blood. The dog was treated with doxycycline (~6.4 mg/kg PO twice daily; Vibramycin, Pfizer) and enrofloxacin (~13 mg/kg PO once daily; Baytril, Bayer) for a total of 6 weeks as well as atovaquone (~13.6 mg/kg PO 3 times daily; Mepron, GlaxoSmithKline, Research Triangle Park, North Carolina) and azithromycin (~10.3 mg/kg PO once daily; Zithromax, Pfizer) for 10 days for the potential of coinfections with *Babesia* spp. and *B*

rochalimae. In conjunction with this treatment, the lameness, thrombocytopenia, and fever resolved. As of November 2019, the dog was clinically normal.

3.4 | Dog 4

A 2-year-old-male intact German Shepherd Dog from Texas was examined for acute onset, unilateral epistaxis. Hematological abnormalities included anemia (HCT 34.5%), leukocytosis (WBC count 18 600/μL) and thrombocytopenia (platelet count of 106 000/μL). *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, and *Rickettsia* PCR assays were negative. Blood and urine cultures were negative for bacterial and fungal growth. Echocardiographic abnormalities included vegetative lesions involving the mitral and aortic valves. Thoracic radiographs identified 3 pulmonary nodules; a 9.4-mm nodule in the ventral aspect of the right cranial lung lobe, a 4.8-mm rounded nodule in the right middle lung lobe, and 4.9 mm rounded nodule overlying the distal aspect of the second rib. The dog was treated with aspirin, lidocaine, omeprazole, famotidine, sucralfate, ampicillin (22 mg/kg IV every 8 hours), and enrofloxacin (10 mg/kg IV every 24 hours; Baytril, Bayer). The dog was IFA seroreactive to *B henselae* (1 : 512) and *B koehlerae* (1 : 128) antigens. Antibiotic treatment was changed to azithromycin (8 mg/kg PO every 24 hours; Zithromax, Pfizer) for 7 days followed by administration every 48 hours and doxycycline (8 mg/kg PO every 12 hours; Vibramycin, Pfizer). The dog continued to do well during antibiotic administration; however, 1 month after initial hospitalization, thoracic radiographs were consistent with CHF; because of poor prognosis, the dog was euthanized approximately 46 days after initial evaluation. After necropsy, aortic and mitral valve endocarditis with dystrophic mineralization of the valves was confirmed histopathologically; no bacteria were visualized. *Bartonella rochalimae* and *B vinsonii* subsp. *berkhoffii* genotype III DNA was PCR-amplified from a frozen EDTA blood sample and a portion of the aortic valve, respectively.

3.5 | Dog 5

A 4-year-old-male intact German Shepherd Dog from Florida was examined because of coughing and increased respiratory effort. A grade IV/VI left basilar systolic and grade III/VI left basilar diastolic murmur were newly auscultated. The dog was anemic (HCT 33%). Platelet numbers were reportedly within normal limits; however, a numerical platelet count was not reported. The dog was IFA seroreactive to *B henselae* (1 : 8192), *B vinsonii* subsp. *berkhoffii* (1 : 2048), *B koehlerae* (1 : 8192), and *R rickettsii* (1 : 64). *Leishmania* IFA, SNAP 4DX ELISA results and *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, and *Rickettsia* PCR assays were negative. *Bartonella rochalimae* DNA was PCR-amplified and sequenced from the dog's blood. Echocardiographic abnormalities included vegetative lesions involving the aortic and mitral valves, severe aortic valve regurgitation, and mild mitral valve regurgitation. Thoracic radiographs were consistent with left sided congestive heart failure. Dog 5 was treated with ampicillin-sulbactam (30 mg/kg IV every 8 hours) and enrofloxacin (10 mg/kg IV every 24 hours; Baytril, Bayer) in addition to standard treatment for left-sided congestive heart failure. The owner elected euthanasia shortly after hospitalization.

3.6 | Dog 6

A 3-year-old-male intact Great Pyrenees from Texas was examined because of recent onset generalized seizures. The dog was anemic (HCT 33.3%), but the platelet count was normal (platelets 242 000/μL). Serum globulins were within the reference interval at 3.4 g/dL. The dog was IFA nonseroreactive to *Babesia canis*, *B henselae*, *B vinsonii* subsp. *berkhoffii*, *B koehlerae*, *E canis*, and *R rickettsii* antigens. *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, and *Rickettsia* PCR assays were negative. *Bartonella rochalimae* DNA was PCR amplified from the dog's blood. Thoracic radiographs were reportedly unremarkable. Cerebrospinal fluid (CSF) was acellular with no evidence of inflammation, neoplasia, or infectious agents. *Bartonella* alpha proteobacteria growth medium culture (ePCR, Galaxy Diagnostics, Morrisville, North Carolina) of CSF did not result in isolation or amplification of *B rochalimae* DNA. Anticonvulsant treatment with levetiracetam was initiated after the first reported seizure and continued to this day. The dog was treated empirically with doxycycline (Vibramycin, Pfizer) and clindamycin (Cleocin, Pfizer) for possible CNS infection from October 2014 until February 2015. Dog 6 reportedly has a good quality of life as of March 2019, 5 years after initial seizure.

3.7 | Dog 7

A 2-year-old-male castrated Boxer from Texas was examined because of lameness and intermittent fever. Hematological abnormalities included anemia (HCT of 32%, lower reference interval 31%) and leukocytosis (WBC 23 300/μL). The platelet count was normal (400 000/μL). Serum globulins were within the reference interval at 3.5 g/dL. Aerobic urine culture did not grow bacteria. Serum was not submitted for infectious disease testing. Echocardiographic abnormalities included an aortic valve vegetative lesion, severe aortic insufficiency, moderate aortic stenosis, and severe left ventricular dilatation. Treatment for endocarditis consisted of amoxicillin/clavulanate and enrofloxacin (unknown doses; Baytril, Bayer). One month after the initial evaluation, an echocardiogram documented marked progression of the left ventricular dilatation. The owner elected euthanasia. At postmortem, aerobic culture of joint fluid grew *Escherichia coli*. Aerobic culture of the aortic valve grew multiple bacteria (a gram-negative nonfermenter; 2 phenotypically different *E coli*, *Enterococcus* spp., a gram-positive pleomorphic rod, and *Enterobacter* spp.). *Bartonella rochalimae* DNA was PCR-amplified from the aortic valve.

3.8 | Dog 8

A 14-month-old-male intact German Shepherd Dog from Virginia was tested for vector borne disease infection as routine working dog

screening. The dog originated from Hungary and was tested 7 days after arrival in the United States. At the same time, a CBC, serum biochemical profile, and urinalysis were normal except for thrombocytopenia (42 000/μL, lower reference interval 200 000/μL) and eosinophilia (1160/μL, upper reference interval 800/μL). Fecal floatation and heartworm antigen tests were negative. The dog was IFA seroreactive to *B henselae* (1 : 128) and *B koehlerae* (1 : 64). *Leishmania* IFA, SNAP 4DX ELISA results and *Anaplasma*, *Babesia*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, and *Rickettsia* PCR assays were negative. *Bartonella rochalimae* DNA was PCR-amplified and sequenced from the dog's blood and an isolate was obtained. The dog is being treated with doxycycline and enrofloxacin.

4 | DISCUSSION

In this case series, we document a spectrum of clinical presentations and mild hematological abnormalities in association with *B rochalimae*, a *Bartonella* spp. that to date has only been implicated in association with IE.[16,18] In addition to IE, dogs in this case series experienced clinical manifestations such as lameness, antibiotic responsive polyarthropathy and seizures, complaints that have not previously been reported in association with *B rochalimae*. Infection was confirmed in 1 dog as a result of routine vector borne screening. It is impossible to prove causation between this bacterium and the various presenting complaints in these dogs on the basis of a retrospective case series. However, on a comparative medical basis, *Bartonella* spp. have been increasingly associated with a spectrum of cardiovascular, neurological, and rheumatologic presentations in human patients.[20,28,33] Thus, prospective case controlled studies are needed to assess the frequency of each of these associations in dogs. Despite the severity of illness, these relatively young *B rochalimae*-infected dogs had minimal and inconsistent hematologic, biochemical, and urinalysis abnormalities, thus limiting the utility of a minimum laboratory database when bartonellosis is suspected. Although an uncommon hematological abnormality, thrombocytopenia (4/8) or platelet counts in the low reference interval (1/7) occurred in a subset of *B rochalimae* infected dogs. Also, despite a diagnosis of endocarditis in 5 dogs, only mild neutrophilia, without a regenerative left shift was documented. Of the 6 dogs with serum globulins available for review, none had values below the reference interval. Of these 6 dogs, 2 had serum globulins above the reference interval and 3 were within the reference interval. This finding is inconsistent with reports of hypoglobulinemia in association with *Bartonella* spp. infection in dogs.[34] This inconsistency is possibly because the number of cases in this series is insufficient to make predictions about a larger population. Another consideration is that serum globulins might be affected differently in dogs infected with *B rochalimae* when compared to other *Bartonella* spp. The clinical and microbiological relevance of the bacteria grown from the aortic valve or joint fluid of dog 7 could reflect postmortem contaminants or concurrent infection with *B rochalimae* and opportunistic bacteria.[35] As dog 6 was bacteremic, but not *Bartonella* spp. seroreactive when first examined for generalized seizures, convalescent serology would have been useful to determine if the dog might have been recently infected.

This case series further supports a role for *B rochalimae* as a cause of blood culture-negative IE. Infectious endocarditis is an uncommon (incidence 0.05% of cases presented annually to a veterinary medical teaching hospital) but frequently detrimental disease with a reported mortality rate in confirmed cases of 56% and a median survival time (MST) of 54 days.[13] While morbidity and death are commonly associated with IE, definitive diagnosis is often elusive because of nebulous clinical signs such as lethargy, fever, and sequelae such as lameness secondary to polyarthritis, glomerulonephritis, and thromboembolic disease.[14,15] Infectious endocarditis occurs when the cardiac endothelium becomes damaged and susceptible to microbial colonization. The most common locations of IE caused by organisms other than *Bartonella* spp. in dogs are the mitral and aortic valves.[13] Interestingly, *Bartonella* spp. most often colonize the aortic valve of both dogs and humans.[12,14,36] There are a vast number of organisms reported in association with IE, but the disease is most frequently associated with *Staphylococcus* spp., *Streptococcus* spp., and *E coli*.[14] Less commonly, IE has been associated with organisms such as *Corynebacterium*, *Proteus*, *Enterobacter*, *Pasteurella*, *Actinomyces turicensis*, and an Actinomyces-like bacterium.[14,17] In recent years, *Bartonella* spp. have emerged as a frequent cause of culture-negative IE.[16,20] The diagnosis of IE can be achieved by historical analysis of clinical signs in conjunction with a combination of methods including cardiac auscultation, visualization of vegetative lesions by echocardiography, aerobic/anaerobic blood cultures, PCR, serology, and valve histopathology.[14,19] Whereas some causative organisms of IE can be isolated with routine culture techniques, *Bartonella* spp. are, as a group, fastidious organisms that more commonly result in blood-culture-negative endocarditis.[20] As a result, the true prevalence of *Bartonella* associated IE is likely underestimated. Previously, we reported IE in military working dogs infected with *B vinsonii* subsp. *berkhoffii* genotype III, 1 of which was coinfected with *B rochalimae*.[18] Although biased by ongoing testing of various working dogs in our diagnostic laboratory, it is of interest that 4 German Shepherd working dogs in our study were infected with *B rochalimae*. This might reflect an IE predilection in large breed working dogs, common environmental exposures, bacterial proliferation and heart valve localization because of working dog stressors, or as yet other uncharacterized factors.

Given the fastidious nature of *Bartonella* spp., isolation, culture, and definitive diagnostic identification of infections with members of this genus is often difficult. Identification of novel clinical presentations associated with *Bartonella* spp. via serology is likely compromised because of the limited number of *Bartonella* spp. antigens represented on commercially available panels and the insensitivity of *Bartonella* spp. IFA assays.[37] The comprehensive vector-borne disease panel performed by the NCSU-CVM-VBDDL contains antigens for *B henselae*, *B koehlerae*, and *B vinsonii* subsp. *berkoffii*. In this series, 4 of 5 dogs that had serology performed were seroreactive for all 3 *Bartonella* spp. antigens, of which 3 were diagnosed with IE. This may represent serological cross reactivity due to frequent showering of bacteria from the IE heart valve in conjunction with IFA recognition of a broader spectrum of shared antigens.[38-40] Although difficult to confirm microbiologically, coinfection with more than 1 *Bartonella* spp. is also a diagnostic consideration given the high rate of coinfection with multiple vector borne pathogens in vectors and hosts.[41,42] Case 8, from which *B rochalimae*

was isolated, had low antibody titers to *B henselae* and *B koehlerae*. Because of substantial genetic diversity among the 38 named *Bartonella* spp., development and validation of PCR assays that are sensitive and specific to the species level have been a formidable challenge for research and diagnostic laboratories.[43] This was illustrated by successful amplification of *B rochalimae* DNA using 1 of the 2 ITS primer sets, as well as the *ssrA* gene target, but no amplification with a different ITS primer set, emphasizing the adage: A negative PCR result does not rule out infection with a specific pathogen in all instances.

Treatment for IE suspected to be associated with organisms other than *Bartonella* spp. generally consists of long-term (at least 6-8 weeks) treatment with broad-spectrum antibiotics; this treatment may be initiated empirically but is ideally guided by blood culture and susceptibility.[14] The dogs in our study were treated with various iterations of the current treatment recommendations for bartonellosis, including extended courses of doxycycline (Vibramycin, Pfizer) and a fluoroquinolone.[44,45]

The prognosis for IE is poor so some dogs diagnosed with IE might be treated with antibiotics for the entirety of their life; the decision on whether or not to discontinue antibiotic treatment is difficult and might be influenced by repeat blood culture, echocardiographic appearance of the heart valve, or CBC changes such as leukocytosis.[14,19] Whereas the prognosis with IE is poor regardless of the affected valve, the prognosis with IE of the aortic valve is grave with a MST of 3 days; this is compared to dogs with IE of the mitral valve (MST 476 days).[14,19] *Bartonella* spp. associated IE has a poorer prognosis, when compared to IE caused by other organisms. This might be associated with incomplete antibiotic elimination of *Bartonella* spp. or the predisposition of *Bartonella* spp. to infect the aortic valve, which is associated with a poorer prognosis when compared to IE affecting the mitral valve, regardless of the inciting bacteria.[14,19,46] Administration of an aminoglycoside at the outset of IE antibiotic treatment has improved prognosis (shorter hospitalization and decreased heart valve replacement surgeries) in human case series.[47]

Whereas this case series provides useful insights into possible clinical and hematological abnormalities associated with *B rochalimae*, it is inherently limited because of the retrospective nature. Limitations included variable detail provided among medical records, variability in diagnostic assessments, and inconsistent treatment regimens. Further research including prospective, case-control studies to evaluate the possible clinical and hematologic manifestations of *B rochalimae* infection are warranted. A prospective study design would allow investigators to perform consistent diagnostic testing in conjunction with a defined treatment regimen for all cases.

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CONFLICT OF INTEREST DECLARATION

B. Q. is a research assistant professor at NC State-CVM, codirector of the NCSU-CVM-VBDDL, and a vector borne disease researcher. E. B. B. codirects the VBDDL and the director of the Intracellular Pathogens Research Laboratory at NC State, and is Chief Scientific Officer at Galaxy Diagnostics, Research Triangle Park, North Carolina.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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