Veterinary Parasitology: Regional Studies and Reports 7 (2017) 19–24

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Case Report

Bartonella henselae, *Bartonella koehlerae* and *Rickettsia rickettsii* seroconversion and seroreversion in a dog with acute-onset fever, lameness, and lymphadenopathy followed by a protracted disease course

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 22 November 2016
Accepted 4 December 2016
Available online 09 December 2016

Keywords:
Bartonellosis
Rickettsiosis
Seroconversion and seroreversion

ABSTRACT

Following recent tick exposure in Arkansas, a 2-year-old, female spayed Labradoodle was examined because of a one-week history of lethargy and shifting-leg lameness. The dog was febrile, had prominent lymph nodes, dull mentation, a stiff gait, and left forelimb lameness. Thrombocytopenia was the only initial hematological or biochemical abnormality. Despite treatment with doxycycline for suspected Rocky Mountain spotted fever, the dog continued to have waxing and waning clinical signs including inappetence, fever, shifting-leg lameness, lymphadenopathy, splenomegaly, and weight loss in association with moderate to severe hematological abnormalities, including anemia, thrombocytopenia, neutrophilia, and monocytosis. Sequential serological testing confirmed *Bartonella henselae*, *Bartonella koehlerae* and *R. rickettsii* seroconversion. Doxycycline, enrofloxacin and clarithromycin were administered in sequential combination for treatment of rickettsioses, *B. henselae* and *B. koehlerae*. Prednisone, thyroid supplementation and other drugs were administered to elicit symptomatic improvement. Based upon seroreversion, and the eventual resolution of all clinical and hematological abnormalities, therapeutic elimination of all three pathogens was seemingly achieved. Whether cortisol insufficiency due to adrenal exhaustion syndrome or post-infectious immune-mediated sequelae contributed to the symptoms and pathophysiological abnormalities reported in this dog was not determined, but are considerations for future cases.

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1. Introduction

Rocky Mountain spotted fever (RMSF), a tick-borne illness caused by *Rickettsia rickettsii*, is characterized by an acute-onset febrile illness that begins to resolve within 12–48 h following doxycycline administration (Breitschwerdt et al., 1988). Duration of rickettsemia in untreated dogs is believed to be days to weeks and immunity following non-fatal infection is long-lived (Breitschwerdt et al., 1990). In contrast, most dogs with PCR or culture confirmed bartonellosis have protracted illnesses accompanied by substantial variations in symptomatology and a less predictable response to antibiotic therapy (Perez et al., 2011; Breitschwerdt et al., 2010). Because *Bartonella* spp. are difficult to detect with current microbiological techniques and because the bacteria can induce a longstanding and potentially a relapsing pattern of bacteremia, it has been difficult to determine the mode(s) of transmission, as well as the onset or duration of infection in sick dogs (Perez et al., 2011; Breitschwerdt et al., 2010).

This report describes an acute-onset febrile illness following tick exposure in a dog from Arkansas. In contrast to the expected rapid resolution post-doxycycline treatment response for RMSF, this dog subsequently developed a chronic, waxing and waning fever, skin lesions and moderate to severe hematological abnormalities during the next year. After *Bartonella henselae*, *Bartonella koehlerae* and *R. rickettsii* seroconversion was convincingly documented, the dog was treated with long-term antibiotics for bartonellosis in conjunction with various symptomatic therapies. Subsequently, there was gradual improvement in clinical signs and hematological abnormalities. Documentation of seroreversion (post-treatment antibodies were not detectable at 1:16 and 1:32 screening dilutions in convalescent sera) in conjunction with resolution of all clinical signs and hematological abnormalities supported therapeutic elimination of the rickettsioses and bartonelloses.

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http://dx.doi.org/10.1016/j.vprsr.2016.12.002
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Table 1
Sequential body weight, and selected complete blood count and serum biochemical test results for a dog with acute-onset illness and seroconversion to *Bartonella henselae*, *Bartonella koehlerae* and *Rickettsia rickettsii* antigens.

Parameters (reference intervals)	Testing dates									
	5/12/2014	7/25/2014	8/13/2014	8/24/2014	10/9/2014	11/19/2014	11/24/2014	11/28/2014	12/8/2014	12/15/2014
Weight (kg)	25.5	19.9	21.6	22.7	24.7	24.8	24.6	ND	22.5	ND
HCT (37.3–61.7 K/μL)	42.3	36.7	29.2	37.4	33.2	27.2	23.6	20.0	22.5	22.8
WBC (5.05–16.76 K/μL)	10.91	24.48	29.58	12.13	13.82	51.38	42.72	57.24	30.08	37.34
Neutrophil (2.95–11.64 K/μL)	7.60	18.63	25.62	7.62	10.29	44.44	36.26	45.40	25.34	33.30
Monocyte (0.16–1.12 K/μL)	1.06	2.02	2.69	1.29	1.18	4.32	4.05	8.04	2.83	1.84
Platelets (148–484 K/μL)	135	64	189	332	204	86	51	45	159	257
Albumin (2.3–4.0 g/dL)	3.0	2.2	NT	NT	NT	2.8	NT	NT	NT	NT
Globulin (2.5–4.5 g/dL)	3.6	3.9	NT	NT	NT	3.8	NT	NT	NT	NT
Creatinine (0.5–0.8 mg/dL)	0.9	0.5	NT	NT	NT	0.6	NT	NT	NT	NT
Serum ALP (23–212 U/L)	134	717	NT	NT	NT	441	NT	NT	NT	NT

ND - not done, NT - not tested, ALP – alkaline phosphatase, HCT – hematocrit, WBC – white blood cells.

2. Materials and methods

2.1. Animal and clinical investigation

On 12 May 2014, a 2-year-old, 25.5 kg, female spayed Labradoodle was evaluated at Faithful Friends Animal Clinic for a one week history of lethargy and shifting leg lameness. Owners reported historical flea infestations and had recently removed ticks from the dog that resided in an urban home with trips to a rural lake house. Physical examination abnormalities included fever (39.5 °C), prominent superficial cervical and popliteal lymph nodes, dull mentation, neck pain, stiff gait, and left forelimb lameness, without palpable joint effusion. Thrombocytopenia was the only complete blood count (CBC) abnormality and serum biochemical results were within reference ranges (Table 1). *Dirofilaria immitis* antigen, *Ehrlichia canis*, *Anaplasma* spp., and *Borrelia burgdorferi* antibodies were negative.[1] Historically, the dog received monthly heartworm[2] and tick[3] preventive medications. Abdominal radiographs identified splenomegaly and diffuse gastrointestinal gas. Initial differential diagnoses included panosteitis, Rocky Mountain spotted fever (RMSF), and immune-mediated polyarthritis. Treatment was initiated for suspected RMSF (doxycycline 9.8 mg/kg q12h PO and deracoxib 1.5 mg/kg q12h PO).

Appetite and ambulation improved within 48 h; however, three days later the dog remained febrile (39.9 °C). Physical examination findings remained unchanged. Tramadol (4 mg/kg q12h PO) was administered for enhanced pain control. Four days later, the dog was more active, less painful, and afebrile (38.6 °C); however, nickel-sized ulcerated skin lesions (cytology mixed inflammatory infiltrate with no visible microorganisms) had developed in the left flank. On May 21st, ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood and serum specimens were submitted for a canine vector borne disease (CVBD) serology/PCR panel.[4] Serology and PCR results were negative, except a *Rickettsia rickettsii* IFA antibody titer of 1:128 (Table 2).

Despite continuous administration of doxycycline and deracoxib, waxing and waning symptoms including lethargy, poor appetite, lymphadenopathy and stiffness persisted between May 12th and June 27th. Also, circular, reddened dermal areas would appear and spontaneously resolve. Due to the unexpected, incomplete response to doxycycline for treatment of RMSF, the attending veterinarian consulted with the corresponding author. Based upon persistent fever, lymphadenopathy, thrombocytopenia, and the incomplete doxycycline treatment response, co-infection with a *Babesia* or *Bartonella* spp. became diagnostic considerations. On July 1st, a repeat CVBD serology/PCR panel documented *B. henselae*, *B. koehlerae* and *R. rickettsii* seroconversion. *Bartonella* alpha Proteobacteria growth medium (BAPGM) enrichment blood culture/PCR, performed as previously described, was negative.[3]

By July 9th, clinical signs worsened (cyclical fever, poor appetite, lethargy), enrofloxacin[5] (5 mg/kg SQ) was administered followed by 3 mg/kg q12h PO in conjunction with doxycycline continuation (doxycycline 9.8 mg/kg q12h PO) for treatment of bartonellosis. Four days later, multifocal patches of erythema with occasional small papules, comedones and follicular casts were detected on the ventral abdomen (Fig. 1). These lesions were different from the focal ulcerative lesions observed at illness onset. A biopsy taken from a single patch revealed only lympho-plasmacytic perivascular dermatitis. To further confirm seroconversion, repeat serological testing[4] documented progressively increasing *B. henselae*, *B. koehlerae*, and *R. rickettsii* antibody titers. *Bartonella* and *Rickettsia* DNA was not amplified by PCR from the skin biopsy.

On July 23rd, when examined by the attending veterinarian prior to hospital-based boarding, the dog had lost 5.6 kg body weight during the previous two months, was lethargic, poorly responsive, muscle wasted, and would only eat with coaxing. During boarding at the veterinary hospital, cyclical fevers were documented. Mirtazapine (1.3 mg/kg PO q 24h), Vitamin B12 500 mcg SQ injection were administered for appetite stimulation. CBC abnormalities included anemia, thrombocytopenia and leukocytosis characterized by neutrophilia and monocytosis (Table 1). Serum biochemical abnormalities included hypoalbuminemia, elevated serum alkaline phosphatase (ALP) activity, and hyperphosphatemia. On July 26th, the dog was recumbent with a resting HR > 180 and bounding pulses. Deracoxib was discontinued, dexamethasone was administered subcutaneously and prednisone was administered (1 mg/kg q12h PO) for 3 days, followed by weekly dose reductions to 0.25 mg/kg prednisone EOD. The enrofloxacin dose was increased (5 mg/kg PO q 24h) and doxycycline (9.8 mg/kg q12h PO) was continued. Following discharge, the dog neither worsened nor improved significantly. Cyclic fevers continued with intermittent left foreleg lameness. The dog would eat only after morning prednisone administration. Doxycycline, enrofloxacin and tramadol were continued, the prednisone dose was tapered and an oral acaracide was prescribed for tick control.[6]

On October 9th, after reducing prednisone administration to 0.25 mg/kg EOD for 10 days, the dog was reexamined due to worsening lethargy and lameness. Physical examination abnormalities included fever (39.7 °C) and left hindlimb lameness with otherwise normal ambulation. Anemia persisted, whereas the neutrophil, monocyte and platelet counts were within laboratory reference ranges. *B. henselae*, *B.*

[1] SNAP® 4Dx® PLUS ELISA, IDEXX Laboratories, Westbrook, Maine.
[2] Heartgard Plus, Merial Inc. Atlanta, GA.
[3] Frontline Plus, Merial Inc. Atlanta, GA.
[4] North Carolina State University, College of Veterinary Medicine Vector Borne Disease Diagnostic Laboratory, (NCSU-CVM-VBDDL) Vector Borne Disease Serology/PCR panel includes the SNAP® 4Dx® PLUS ELISA[1], *Babesia*, *Bartonella*, *Ehrlichia* and *Rickettsia* sp. immunofluorescence assays (IFA) and *Anaplasma*, *Babesia*, *Bartonella*, *Ehrlichia*, hemotropic *Mycoplasma* and *Rickettsia* genus PCR assays.
[5] Baytril, Bayer Animal Health, Shawnee KS.
[6] NexGard, Merial, LTD. Atlanta, GA.

12/22/2014	12/29/2014	1/19/2015	3/2/2015	4/7/2015	4/29/2015	6/25/2015	7/17/2015	8/10/2015	3/1/2016
ND	20.5	20.9	22.1	ND	25.5	23.3	21.9	24.6	29
25.2	26.2	28.0	24.5	30.8	33.7	25.5	29.7	36.5	39.3
20.39	12.84	16.41	26.23	14.54	20.83	19.89	18.08	9.51	12.2
16.78	10.77	11.40	20.45	9.28	16.41	14.47	12.79	5.67	9.44
1.23	0.72	2.24	2.14	1.15	0.97	1.24	1.20	0.61	0.67
416	224	225	212	454	320	249	297	317	325
NT	NT	NT	NT	NT	NT	2.2	2.3	NT	3.1
NT	NT	NT	NT	NT	NT	4.7	5.1	NT	3.3
NT	NT	NT	NT	NT	NT	0.6	0.6	NT	1.0
NT	NT	NT	NT	NT	NT	188	223	NT	59

koehlerae and *R. rickettsii* antibody titers had decreased. Due to the persistence of fever and lameness, clarithromycin (10 mg/kg PO **q12h**) was added to the doxycycline and enrofloxacin treatment regimen on October 14th, and prednisone continued (0.25 mg/kg every other day) as previously directed.

On November 19th, the dog was re-examined due to waxing and waning fever (rectal temperatures while hospitalized ranged from 39.6 °C to 40.6 °C). Specifically, rectal temperatures in the morning exceeded PM temperatures (11/25 AM 40.6 °C, PM 38.1 °C, 11/26 AM 40.6 °C, PM 38.7 °C, 11/27 AM 40.5 °C, PM 38.9 °C). Also, between November 19th and 28th, there was a progressive decrease in hematocrit and platelet numbers and a substantial increase in neutrophils and monocytes. The only serum biochemical abnormality was elevated ALP activity. Antibiotic administration ceased. Due to the ongoing and unexplained disease progression, fluconazole (8 mg/kg PO q 12h) was started empirically, the prednisone dose was increased (0.5 mg/kg PO q24h), to determine if the clinical signs were steroid responsive. Blastomycosis, Histoplasmosis, and Coccidioidomycosis serology and a Candida PCR were negative.[7] Fluconazole was discontinued. Thoracic and abdominal radiographs and an urinalysis were unremarkable. When the prednisone dose was reduced to 0.25 mg/kg PO q24hr on 11/27, the dog became lethargic and anorexic, as reported previously by the owner. Therefore the prednisone dose was increased (1 mg/kg PO AM, 0.5 mg/kg PO PM). Cerenia[8] (3 mg/kg PO q24h) was administered for inappetence. The prednisone dose was again tapered weekly to 0.25 mg/kg q24hr.

Between December 8th 2015 and January 19th 2016, serial CBCs identified a gradual increase in hematocrit, a progressive decrease in neutrophils and monocytes and normalization of thrombocytes. On December 15th 2015, Vitamin B12 (500 IU SQ) and a probiotic[9] (300 g PO SID) were administered. By January 2016, the dog's appetite had improved, the dog had gained two pounds body weight, the owner reported improved ambulation, no lameness and increased activity. The dog was re-examined March 2nd due to development of seborrhea and two crusted skin lesions. Thyroid function testing[10] documented low total thyroxine (TT4), total triiodothyronine (TT3), free thyroxine (FT4), free triiodothyronine (FT3), normal thyroid stimulating hormone (TSH) indicative of euthyroid sick syndrome. Serum folate concentration[11] (6.2 μg/L, 7.7–24.4) was decreased and cobalamin was normal. Treatment with weekly subcutaneous Vitamin B12 (500 mcg) injections was reinstated and Soloxine[12] (0.02 mg/kg q12hr PO) was administered. Prednisone (0.25 mg/kg q24hr) and the probiotic[9] were continued. Sequential complete blood counts between April and August 2015 documented gradual resolution of the anemia, thrombocytopenia, neutrophilia and monocytosis. In May 2015, *B. henselae*, *B. koehlerae* and *R. rickettsii* antibodies were not detectable. As of August 12, 2016, the dog was maintained on thyroxine (0.02 mg/kg BID PO), and heartworm and tick preventives monthly. Body weight has increased from 20 kg to 29 kg. The dog remains healthy, extremely active, and has returned to all pre-illness activities and behaviors with no skin lesions, febrile episodes, or lameness within the previous 18 months.

3. Discussion

Medically unique aspects of this dog's illness included infection with *Bartonella* and *Rickettsia* spp. as documented by seroconversion and seroreversion, cutaneous lesions that spontaneously appeared and disappeared, and protracted clinical and hematological abnormalities that persisted during and following an extended antibiotic regimen for bartonellosis. Whether infection with these three organisms occurred simultaneously or alternatively, the rickettsial infection resulted in recrudescence of occult *B. henselae* and *B. koehlerae* infections could not be determined. When using serology to diagnose an acute infection with *R. rickettsii*, a four-fold rise in antibody titer between the acute and convalescent serum sample is required (Breitschwerdt et al., 1988). In this dog, *B. henselae*, *B. koehlerae* and *R. rickettsii* antibodies sequentially increased and subsequently decreased during and following antibiotic therapy, supporting co-infection with these three bacteria. Seroconversion kinetics is pathogen dependent with more virulent pathogens inducing more rapid seroconversion. *Rickettsia rickettsii* is considered the most virulent tick-transmitted bacterial pathogen in North America, which could explain the slightly earlier detection of *Rickettsia* as compared to *Bartonella* antibodies in this dog. As infection with any member of the spotted fever group (SFG) rickettsiae induces cross reactivity to *R. rickettsii* antigens, it is possible that this dog was infected with *Rickettsia parkeri* (which induces eschars in humans) or another rickettsiae. Cross reactivity between species is an unlikely explanation for these serological results as dogs experimentally infected with *R. rickettsii* do not develop cross reactive antibodies to *Bartonella* spp. antigens (Hegarty et al., 2014). Similarly, dogs experimentally infected with *B. henselae*, and *B. vinsonii* subsp. *berkhoffii* and naturally-infected with *B. koehlerae* develop a species specific antibody response, without cross reacting antibodies to *Rickettsia* or other *Bartonella* species (Balakrishnan et al., 2013). In contrast to the seroconversion documented in this dog, other studies have found that approximately three quarters of *B. henselae*-infected dogs (PCR positive from blood, tissues, or BAPGM enrichment blood or tissue culture) are *B. henselae* seronegative by immunofluorescent antibody testing (Perez et al., 2011).

In humans, rash is a predominant feature of Rocky Mountain spotted fever, whereas *R. rickettsii* infection infrequently causes a rash in dogs (Gasser et al., 2001). Because *R. rickettsii* is a highly virulent pathogen

[7] IDEXX Laboratories, Sacramento CA.
[8] Zoetis Animal Health, New York NY.
[9] Conklin Fastrack Canine Microbial Supplement, Shakopee, MN.
[10] Michigan State University Clinical Endocrinology Laboratory, East Lansing, MI.
[11] Gastrointestinal Laboratory, Texas A&M University, College Station Texas.
[12] Virbac Animal Health, Fort Worth, TX.

Table 2
Sequential serology, PCR and BAPGM ePCR test results for a dog with acute-onset illness and seroconversion to *Bartonella henselae*, *Bartonella koehlerae* and *Rickettsia rickettsii* antigens.

Date	SNAP® 4Dx® PLUS ELISA	*Bartonella henselae* serology (IFA) titer	*Bartonella vinsonii* serology (IFA) titer	*Bartonella koehlerae* serology (IFA) titer	*Bartonella* PCR from blood	*Bartonella* PCR from BAPGM enrichment culture	*Rickettsia rickettsii* serology (IFA) titer	*Rickettsia* PCR from blood
5/12/14	Neg	NA	NA	NA	NA	NA	NA	NA
5/21/14	Neg	<1:16	<1:16	<1:16	NA	NA	1:128	NA
7/1/14	NT	1:256	<1:16	1:1024	Neg	Neg	1:512	Neg
7/13/14	NT	1:1024	<1:16	1:2048	Neg	Neg	1:1024	Neg
10/13/14	NT	1:128	1:32	1:16	Neg	Neg	1:64	Neg
5/19/15	Neg	<1:16	<1:16	<1:16	Neg	Neg	<1:16	Neg
6/25/15	Neg	NA	NA	NA	NA	NA	NA	NA
3/2/16	Neg	<1:16	<1:16	<1:16	Neg	Neg	1:64	Neg

NA: blood and/or serum not available for testing.
NT: test not performed.
SNAP® 4Dx® PLUS Neg: no colorimetric reactivity for *Anaplasma* spp., *Borrelia burgdorferi*, *Ehrlichia* spp. or *Dirofilaria immitis*.
PCR or BAPGM ePCR Neg: *Bartonella* or *Rickettsia* DNA was not amplified from blood or in the case of *Bartonella* spp. following BAPGM enrichment culture.

that induces acute endothelial damage and cell death, rash occurs during the acute phase of the infection and often prior to seroconversion (Gasser et al., 2001). Although atypical for RMSF, the ulcerative lesions that developed in the left flank nine days after doxycycline was administered may have been due to rickettsial vasculitis, an eschar as reported for *Rickettsia parkeri* in the southern United States, or vascular injury (vasculitis) induced by *Bartonella*/*Rickettsia* co-infection (Davidson et al., 1990; Delisle et al., 2016; Friedenberg et al., 2015). Due to the short duration of rickettsemia, the fluctuating rash that occurred 46 days after illness onset was not likely associated with rickettsial infection and was potentially related to bartonellosis. Because of the recent discovery of the genus *Bartonella* as a cause of disease in dogs, few studies have addressed the potential role of these bacteria in association with cutaneous disease manifestations (Breitschwerdt et al., 2010). Panniculitis and cutaneous bacillary angiomatosis have been reported as cutaneous lesions in dogs with bartonellosis (Mellor et al., 2006; Cross et al., 2008; Yager et al., 2010). Also, because *Bartonella* spp. can induce long standing, non-clinical infections in numerous animal species, the role of this genus in disease causation has been difficult to establish (Perez et al., 2013). In addition to the vascular niche, where these bacteria infect erythrocytes, monocytes, pericytes and vascular endothelial cells, a dermal niche involving dendritic and potentially other cells in the skin has been more recently emphasized (Breitschwerdt, 2014). The extent to which the dermal niche is of clinical relevance for cutaneous lesions in animals and humans requires additional study.

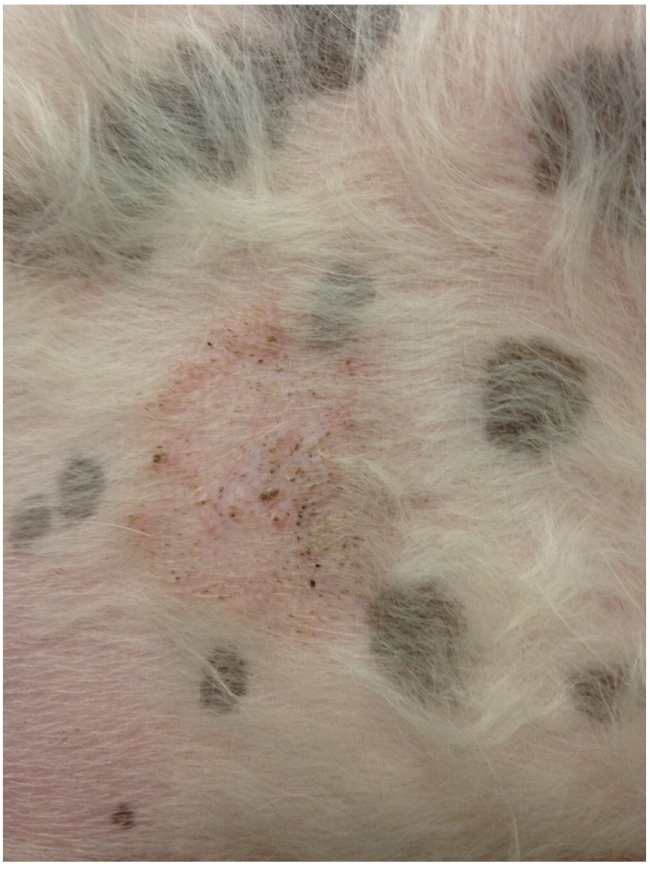

Fig. 1. Multifocal patches of mild erythema with slightly irregular margins on the ventral abdomen. These erythematous areas contain small papules, comedones and darker follicular casts. Papules are predominantly localized at the periphery of the lesion. These lesions would appear and spontaneously disappear. Photograph obtained 46 days after illness onset and following continuous treatment with doxycycline.

Diagnostic confirmation of both acute and chronic vector borne intracellular infections can be clinically challenging (Maggi et al., 2014). In this dog, antibiotic therapy was initiated prior to obtaining pre-treatment blood specimens for *Rickettsia* spp. PCR or *Bartonella* BAPGM enrichment blood culture/PCR. Despite inducing severe organ system pathology and systemic disease manifestations, very low levels of both *Bartonella* and *Rickettsia* spp. DNA are found in peripheral blood, limiting PCR diagnostic sensitivity for diagnosis of bartonellosis and rickettsioses. Previous studies addressing PCR sensitivity in dogs have documented the need to increase *Bartonella* spp. bacterial numbers by enrichment culture prior to PCR testing (Perez et al., 2011; Duncan et al. 2007). Thus it is of substantial diagnostic importance that EDTA-anti-coagulated blood, serum and fresh frozen diseased tissues are obtained prior to initiation of antibiotics. Blood and other tissues obtained for PCR testing should be refrigerated immediately to inactivate degradation of bacterial DNA. In addition, it is important for the clinician to recognize that formalin fixation cross-links and degrades microbial DNA, further compromising the diagnostic usefulness of formalin-fixed paraffin embedded tissues for PCR testing purposes. Based upon a recent study (Balakrishnan et al., unpublished data) of dog skin punch biopsy specimens, formalin fixation for 24 h completed inhibited PCR amplification of 16S rDNA from skin bacterial flora that could be readily amplified from fresh frozen skin samples. Also, as illustrated by this case, concurrent and appropriately timed serological and molecular testing can provide the clinician more comprehensive information upon which to base treatment decisions.

Tick transmission of *R. rickettsii* was the most likely cause of rickettsial seroconversion in this dog. *Bartonella henselae* and *B. koehlerae* are most commonly transmitted by *Ctenocephalides felis*, the common “cat flea”; however, clinical, epidemiological and laboratory tick vector transmission studies increasingly support a potential role for tick transmission of *Bartonella* spp. (Breitschwerdt, 2014). In this case, the owner reported only tick exposure immediately prior to illness onset. If fleas were the source of the *Bartonella* sp. infection, concurrent *Rickettsia felis* transmission would also be a diagnostic consideration. *R. felis*,

transmitted by *C. felis*, has been associated with acute febrile illness in humans throughout "cat" flea endemic regions of the world (Assarasakorn et al., 2012). *R. felis* DNA has also been amplified from the blood of healthy and sick dogs from countries throughout the world; however, evidence to support a pathogenic role in dogs is lacking. Infection with virulent *B. henselae* strains appear to cause fever in cats, dogs, and humans (Drut et al., 2014). Concurrent infection with *B. henselae*, *B. koehlerae* and *R. felis* (or another *Rickettsia* spp.) may have collectively contributed to the acute onset febrile illness in this dog, whereas the persistent infection with two *Bartonella* spp. was thought to be responsible for the chronic, relapsing fever of unknown origin. In geographic locations, where fleas and ticks co-exist, it is important for clinicians to consider near simultaneous or sequential transmission of flea and tick-borne pathogens. Concurrent infections with multiple vector borne pathogens can result in complex disease expression, atypical clinical signs of illness and a spectrum of hematological, biochemical and urinalysis abnormalities (Balakrishnan et al., 2014).

Doxycycline, considered the treatment of choice for rickettsioses, was initiated at the time of initial presentation. Subsequently, doxycycline, enrofloxacin and clarithromycin were used in sequential, combinations to treat *B. henselae* and *B. koehlerae* infection. Despite long-term antibiotic administration, the dog in this case report continued to have waxing and waning clinical signs for 10 months. Based upon CBC trends, there was improvement in the hematocrit, normalization of platelet numbers and a decrease in neutrophils and monocytes after addition of enrofloxacin to the doxycycline treatment regimen during the July–August 2014 time frame. In contrast, the most severe hematological abnormalities (anemia, neutrophilia, monocytosis and thrombocytopenia) were documented in November 2014, approximately 4 weeks after the addition of clarithromycin. Based upon sequential serum biochemistry and urinalysis results, neither the antibiotic combination nor other drugs appeared to induce hepatic or renal adverse drug effects. As an optimal treatment regimen for canine bartonellosis has not been established, these antibiotic combinations were selected on the basis of *in vitro* sensitivity data and evolving microbiological data relative to antibiotic treatment outcomes (Biswas et al., 2010a, 2010b). Based upon decrementing antibody titers (seroreversion), and the eventual resolution of all clinical and hematological abnormalities, we suggest that infection with all three pathogens was eliminated; however, the November 2014 hematological changes and prolonged post-antibiotic recovery time remains unexplained.

In addition to the inherent diagnostic and medical management complexities associated with this dog's illness, financial constraints, temporal, and technical limitations also influenced clinical decisions. The attending clinician and university infectious disease consultant considered post-infectious immune-mediated sequelae for the fever of unknown origin, thrombocytopenia, and polyarthritis. Although immunosuppressive doses of prednisone (1 mg/kg/day) were periodically administered for short time frames, immunosuppression was not a long-term treatment objective, as therapy was directed at an infectious etiology and neither joint taps nor autoimmune testing were pursued. Interestingly, based upon observations by the owner and attending veterinarian, the dog responded clinically to low (physiological or anti-inflammatory) doses of prednisone, which may have been associated suppression of inflammation, a placebo effect, or adrenal exhaustion syndrome, as reported in association with human chronic infectious and non-infectious illnesses (De Jong et al., 2015). The clinical significance of critical illness-related corticosteroid insufficiency (CIRCI) remains controversial in human medicine. Although laboratory testing supported euthyroid sick syndrome, thyroid therapy was initiated due to the dog's dermal lesions, which subsequently resolved.

4. Conclusion

This case report supports a potential role for *Bartonella* spp. as a comorbidity or contributing factor in a poorly defined clinical syndrome characterized by cyclic fever, myalgia, dermal lesions, splenomegaly and weight loss that responded incompletely to doxycycline therapy. Whether this dog's disease manifestations would have been different in the absence of rickettsial infection cannot be determined. Diagnostic specimens, collected prior to initiation of antibiotics for concurrent serological and PCR testing, are recommended to confirm a diagnosis of bartonellosis or rickettsiosis. Sequential, long-term serological testing supported therapeutic and/or immunological elimination of all three infections. Regardless of the pathophysiological mechanisms that contributed to the dog's clinical signs and hematological abnormalities, complete remission of illness was eventually achieved.

Conflict of interest

In conjunction with Dr. Sushama Sontakke and North Carolina State University, Dr. Breitschwerdt holds U.S. Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is the chief scientific officer for Galaxy Diagnostics, a company that provides advanced diagnostic testing for the detection of *Bartonella* species infection.

Funding

This case study was supported in part by the State of North Carolina and donations to the Vector Borne Disease Research Fund, North Carolina State University Veterinary Medical Foundation.

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