

STANDARD ARTICLE

Clinicopathological findings in 41 dogs (2008-2018) naturally infected with *Ehrlichia ewingii*

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Funding information

IDEXX Laboratories, Inc; NC State-CVM Bartonella/Vector Borne Disease Foundation research fund

Background: *Ehrlichia ewingii* is the most seroprevalent *Ehrlichia*-infecting dogs in the southern and mid-western United States. Fever, lameness, and polyarthritis are commonly reported findings in dogs naturally infected with *E. ewingii*.

Objectives: To evaluate clinicopathologic findings in a population of dogs naturally infected with *E. ewingii*.

Animals: Forty-one dogs PCR positive for *E. ewingii* and PCR negative for other targeted vector-borne organisms.

Methods: Retrospective study. Clinical and clinicopathologic data including physical examination findings, CBC, serum biochemistry, urinalysis (UA), symmetric dimethylarginine (SDMA), and vector-borne disease diagnostic results were reviewed.

Results: Frequent clinical diagnoses other than ehrlichiosis (28/41; 68.3%) were renal disease (7/41; 17.1%) and immune-mediated hemolytic anemia (IMHA) (6/41; 14.6%). The most frequent physical examination finding was joint pain (14/41; 34.1%). Prominent hematologic and biochemical abnormalities included abnormal lymphocyte counts (22/36; 61.1%); neutrophilia (21/37; 56.8%); increased alkaline phosphatase (20/35; 57.1%) and alanine transaminase (14/35; 40%) activities; and increased SDMA concentration (11/34; 32.4%). Urinalysis abnormalities included proteinuria (20/27; 74%), most with inactive sediments (16/20; 80%). Dogs were seroreactive by *Ehrlichia canis* immunofluorescence assay (IFA; 17/39; 43.6%) and *Ehrlichia* ELISA (34/41; 82.9%). Seroreactivity by IFA for other vector-borne pathogens included *Bartonella* (1/39; 2.6%), *Rickettsia rickettsii* (spotted-fever group rickettsiae) (12/39; 30.8%), and *Borrelia burgdorferi* by ELISA (1/41; 2.4%).

Conclusions and Clinical Importance: Renal disease, IMHA, proteinuria, neutrophilia, abnormal lymphocytes, and increased liver enzyme activities were common in this group of *E. ewingii*-infected dogs. Studies are needed to determine if *E. ewingii* contributes to comorbidities or is a precipitating factor in clinical syndromes in persistently infected dogs.

KEYWORDS

canine, granulocytic ehrlichiosis, tick-borne

Abbreviations: A-CKD, acute on chronic kidney disease; CGE, canine granulocytic ehrlichiosis; CKD, chronic kidney disease; CLL, chronic lymphocytic leukemia; CME, canine monocytic ehrlichiosis; CVBP, canine vector-borne pathogen; CVM, College of Veterinary Medicine; IFA, indirect fluorescent antibody; IMHA, immune-mediated hemolytic anemia; ITP, immune-mediated thrombocytopenia; NCSU, North Carolina State University; NLR, neutrophil-to-lymphocyte ratio; SDMA, symmetric dimethylarginine; UP/C, urine protein : creatinine ratios; VBDDL, Vector-borne Disease Diagnostic Laboratory.

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1 | INTRODUCTION

Ehrlichia ewingii is an obligate intracellular bacterium that predominantly infects granulocytes, causing granulocytic ehrlichiosis in dogs and humans.^{1,2} Acute granulocytic ehrlichiosis in dogs is characterized most often by fever, thrombocytopenia, and joint pain. Of comparative medicine and zoonotic disease importance, granulocytic ehrlichiosis in humans previously has been reported in immunosuppressed patients, but more recent reports document most cases occurring in immunocompetent patients.^{3–8} Clinical abnormalities in human patients often consist of fever, myalgia, headache, nausea, vomiting, acute renal failure, leukopenia, thrombocytopenia, and increased liver enzyme activities.

Using *Ehrlichia* species-specific peptides, recent studies indicate that *E. ewingii* is the most seroprevalent tick-transmitted *Ehrlichia* spp. affecting dogs in the United States.^{9–11} The primary vector for *E. ewingii* is *Amblyomma americanum*, an aggressive tick that feeds on multiple reservoir hosts, including white-tailed deer, reptiles, birds, and dogs.¹² In contrast, *Ehrlichia canis* is vectored by *Rhipicephalus sanguineus* (sensu lato), a tick most often associated with dog kennels and that primarily feeds on canids as reservoir hosts.¹³ Tick ecology and behavior partially may explain differences in *E. canis* (0.8%) and *E. ewingii* (5.1%) seroprevalence rates among dogs in the South and Central regions of the United States.⁹

Based on several studies involving naturally and experimentally infected dogs, canine granulocytic ehrlichiosis (CGE), caused by *E. ewingii*, has been associated with less severe clinical signs relative to canine monocytic ehrlichiosis (CME), caused by *E. canis*.^{11,14–20} Dr. Sidney Ewing initially observed *E. ewingii* as morulae in the neutrophils of dogs with thrombocytopenia and polyarthritis.¹ With the advent of molecular phylogenetics, *E. ewingii* was later identified as a species distinct from *E. canis* based on 16S rRNA gene sequence analyses.² Clinicopathologic abnormalities reported in dogs with CGE include fever, lameness, neurological abnormalities, lymphadenomegaly, peripheral edema, neutrophilic polyarthritis, thrombocytopenia, and leukopenia.^{14–17,19,20} Several of these studies also reported *E. ewingii*-infected dogs with no clinical abnormalities. Dogs experimentally infected with *E. ewingii* after exposure to *A. americanum* continued to harbor *E. ewingii* DNA, indicated by continuous PCR-positive results, for up to 2 years without developing overt clinical disease.¹⁸ It is not clear why a subset of *E. ewingii*-infected dogs develop clinical disease, whereas others immunologically eliminate the infection or remain subclinical and persistently infected. Possible reasons include differences in the overall health status of individual dogs, *E. ewingii* strain variance, the presence of coinfections, or some combination of these factors. Coinfections caused by vector-borne pathogen can produce atypical clinical presentations and increase illness severity. Previous studies of dogs naturally infected with *E. ewingii* did not test for concurrent infections with other vector-borne pathogens, except *Ehrlichia chaffeensis* or *Anaplasma* spp. in some reports.^{11,14–16,18,19} The purpose of our study was to characterize disease manifestations in a large number of naturally infected, *E. ewingii* PCR-positive dogs that were concurrently PCR negative using a panel of other common canine vector-borne pathogens (CVBP).

2 | METHODS

A convenience sample of dogs with PCR-confirmed *E. ewingii* infection was assembled for our study by review of a database containing results from CVBP diagnostic testing performed at the North Carolina State University (NCSU), College of Veterinary Medicine (CVM), Vector-borne Disease Diagnostic Laboratory (VBDDL) between January 1, 2008 and February 7, 2018. Dogs were eligible for study inclusion if (1) the CVBP-PCR panel (Canine vector-borne disease diagnostic panel, Vector Borne Disease Diagnostic Laboratory, NCSU, Raleigh, North Carolina) was *E. ewingii* PCR positive and PCR negative for *E. canis*, *E. chaffeensis*, Panola Mountain *Ehrlichia*, *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., hemotropic *Mycoplasma* spp., and *Rickettsia* spp. and (2) medical data were concurrently available for analysis. In addition, any *E. ewingii* PCR-positive dogs reported as having a recent blood transfusion before PCR testing or that were *Bartonella* PCR positive by *Bartonella* alpha-Proteobacteria growth medium (BAPGM) enrichment culture were excluded. To increase the number of dogs included in the study, a CVBP-PCR panel was performed retrospectively by the VBDDL on specimen submissions that were *E. ewingii* PCR positive but not initially tested for coinfections and for a small set of dogs identified in a previous study that were *E. ewingii* seroreactive by SNAP[®] Multi-Analyte but not PCR tested.¹⁰ To compare SNAP[®]4Dx[®]Plus Test and PCR results, dogs included in our study that did not initially have SNAP[®]4Dx[®]Plus Test results were retrospectively tested using available sera or ethylenediaminetetraacetic acid-anticoagulated whole blood.

Medical records and CVBP-PCR diagnostic test results were reviewed. Patient medical record data included signalment, owner address, historical findings, clinical diagnoses, physical examination findings, CBC, serum biochemistry panel, urinalysis (UA), and results from a CVBP-serology panel (Canine vector-borne disease diagnostic panel, Vector Borne Disease Diagnostic Laboratory, North Carolina State University, Raleigh, NC) that included immunofluorescence assay (IFA) testing for *Babesia canis*, *Babesia gibsoni*, *Bartonella henselae*, *Bartonella koehlerae*, *Bartonella vinsonii berkoferii*, *E. canis*, and *Rickettsia rickettsii* (spotted fever group rickettsia); and SNAP[®]4Dx[®]Plus Test for seroreactivity to *Anaplasma* spp., *Ehrlichia* spp., and *Borrelia burgdorferi* and the presence of *Dirofilaria immitis* antigen. Dogs were not excluded based on seroreactivity to other CVBP.

Except for 4 dogs, CBC and serum biochemistry testing was completed by NCSU, CVM clinical pathology laboratory (Clinical Pathology Department at the NCSU, CVM, Veterinary Hospital, Raleigh, North Carolina). For 3 dogs, serum biochemistry results were generated using after-hours diagnostic equipment, and the remaining dog was tested by a commercial laboratory (Antech Diagnostics, Irvine, California). Results for abnormal variables reported in our study only include values that were above or below respective reference intervals. The CBCs evaluated by NCSU CVM clinical pathology laboratory included identification of abnormal lymphocytes, defined as small to intermediate in size with increased amounts of pale cytoplasm (without a blast appearance). The neutrophil-to-lymphocyte ratio (NLR) was defined as the absolute neutrophil count divided by the absolute lymphocyte count. A NLR

TABLE 1 CVBP serological and *Dirofilaria immitis* antigen results for 41 dogs infected with *Ehrlichia ewingii*, determined by SNAP[®]4Dx[®] Plus Test and IFA using whole-cell antigens from *Babesia canis*, *Babesia gibsoni*, *Bartonella henselae*, *Bartonella koehlerae*, *Bartonella vinsonii berkefeldii*, *Ehrlichia canis*, and *Rickettsia rickettsii*

CVBP	Positive dogs/total tested (%)	SNAP [®] 4Dx [®] Plus Test	IFA titer 1 : 64	IFA titer 1 : 128	IFA titer 1 : 256	IFA titer 1 : 512	IFA titer 1 : 1024	IFA titer 1 : 2048
<i>Anaplasma</i> spp.	0/41	0	NA	NA	NA	NA	NA	NA
<i>B. burgdorferi</i>	1/41 (2.4)	1	NA	NA	NA	NA	NA	NA
<i>Ehrlichia</i> spp.	34/41 (82.9)	34	NA	NA	NA	NA	NA	NA
<i>D. immitis</i>	0/41	0	NA	NA	NA	NA	NA	NA
<i>B. canis</i>	0/39	NA	0	0	0	0	0	0
<i>B. gibsoni</i>	0/23	NA	0	0	0	0	0	0
<i>B. henselae</i>	1/39	NA	0	0	0	1	0	0
<i>B. koehlerae</i>	1/25	NA	0	1	0	0	0	0
<i>B. vinsonii berkefeldii</i>	1/39	NA	0	0	1	0	0	0
<i>E. canis</i>	17/39 (42.4)	NA	5	10	1	1	0	0
<i>R. rickettsii</i>	12/39 (30.3)	NA	3	4	3	0	1	1

IFA titers $\geq 1 : 64$ indicate positive exposure. Seroreactivity was not a basis for exclusion from our study. Abbreviations: IFA, immunofluorescence assay; NA, not applicable.

cutoff of >5 was used, based on a previously published study in healthy dogs.²¹

After study inclusion, symmetric dimethylarginine (SDMA) renal biomarker testing was retrospectively performed by a commercial laboratory (IDEXX Laboratories, Inc, Westbrook, Maine) when archived serum was available.

3 | RESULTS

3.1 | Signalment

Between September 2008 and February 2018, 41 dogs met the inclusion criteria (40 evaluated at a veterinary referral hospital, NCSU Veterinary Hospital and 1 at a private veterinary hospital that submitted clinical data). All dogs resided in North Carolina. Twenty-two dogs were males (18 neutered), and 19 dogs were females (18 spayed). The median age was 9 years (mean, 8.6 years; range, 2.5-13 years), with 7 mixed breeds and 34 purebred breeds, most common being Labrador Retrievers ($n = 4$) and German Shepherd Dogs ($n = 3$). Seasonally, *E. ewingii* PCR-positive dogs were diagnosed during summer (16/41, 39%), fall (11/41, 26.8%), winter (10/41, 24.4%), and spring (4/41, 9.8%).

3.2 | Vector-borne disease diagnostic testing

According to the inclusion criteria, all 41 *E. ewingii* PCR-positive dogs were PCR negative for *E. canis*, *E. chaffeensis*, Panola Mountain *Ehrlichia*, *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., Hemotropic *Mycoplasma* spp., and *Rickettsia* spp. Of the 12 323 canine samples submitted for *Ehrlichia*-specific or CVBP panel PCR testing to the VBDDL between January 1, 2008 and February 7, 2018, 187 (1.5%) dogs were *E. ewingii* PCR positive and PCR negative for a CVBP coinfection. Of those, 45 (24.1%) dogs had medical data available for analysis, but 4 were excluded for various reasons, including 1 dog that received a recent blood transfusion before presentation and 3 dogs with coinfections (2 *Bartonella* positive by BAPGM enrichment culture indicated in the medical record and 1 retrospectively identified

hemotropic mycoplasma infection). Nine (22%) of the 41 *E. ewingii* PCR-positive dogs were retrospectively identified, because testing for this organism was not initially requested by the attending veterinarian. At initial presentation, a CVBP serology panel was requested for 39 of 41 (95.1%) dogs. Of these 39 dogs, 23 (59%) were screened with a complete CVBP-serology panel but because of unavailability of IFA antigens, 14 of 39 (35.9%) dogs did not include *B. gibsoni* and *B. koehlerae* and 2 of 39 (5.1%) dogs did not include *B. gibsoni* (Table 1). With the exceptions of *Bartonella* spp. (1/39; 2.6%), *E. canis* (17/39; 43.6%), and *R. rickettsii* (12/39; 30.8%) IFA seroreactivity, all dogs were IFA seronegative. SNAP[®]4Dx[®] Plus Tests, performed either at the time of initial diagnostic testing or retrospectively, identified 34 of 41 (82.9%) dogs seroreactive to *Ehrlichia* peptides, representing *E. canis*, *E. chaffeensis*, or *E. ewingii* exposure, or some combination of these, and 1 of 41 (2.4%) dogs seroreactive to *B. burgdorferi* C6 peptide.

3.3 | Clinical diagnoses

Medical record data were extracted and reviewed for primary complaints and concurrent clinical findings reported at the time of assessment by the attending clinicians (Figure 1). Of the 41 dogs evaluated, frequent primary diagnoses included renal disease, reported in 7 (17.1%) dogs, and immune-mediated hemolytic anemia (IMHA) reported in 6 (14.6%) dogs. Of the dogs with renal disease, 4 were diagnosed with acute-on-chronic kidney disease (A-CKD), 2 with chronic kidney disease (CKD), and 1 with protein-losing nephropathy (PLN). Kidney disease diagnoses were based on increased serum creatinine concentrations with dilute urine. Protein-losing nephropathy was diagnosed based on proteinuria and ultrasonographic renal abnormalities indicative of chronic nephropathy. These 7 dogs also had SDMA concentrations ≥ 20 $\mu\text{g/dL}$. Of the 6 IMHA dogs, 1 had concurrent A-CKD. Neurological disease was diagnosed in 5 (12.2%) dogs, including 2 dogs with undiagnosed seizures; 1 dog with a cranial nerve neuropathy; 1 dog with a right forebrain lesion attributed to

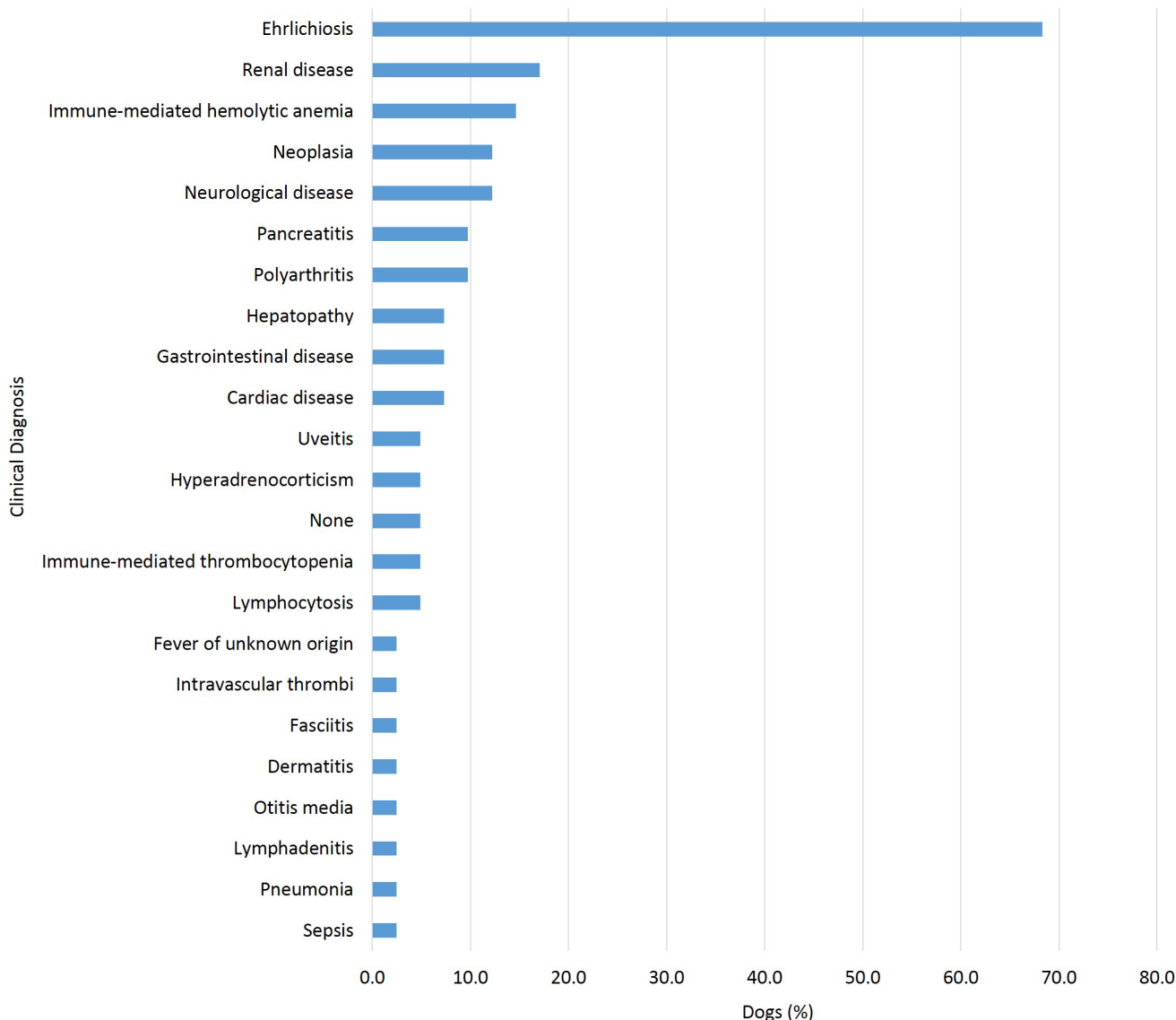


FIGURE 1 Multiple concurrent clinical diagnoses made at the time of assessment by the attending clinicians for 41 *Ehrlichia ewingii* PCR-positive dogs. Clinical diagnoses proportions are organized from highest to lowest

meningoencephalomyelitis, infection, stroke, or neoplasia; and 1 dog with infectious or immune-mediated encephalitis. Neoplasia was diagnosed in 5 (12.2%) dogs, including chronic lymphocytic leukemia (CLL), hemangiosarcoma, brain tumor, nasal tumor, and testicular tumor. Polyarthritis, attributed to *E. ewingii* infection according to the attending clinician, was diagnosed in 4 (9.8%) dogs. Pancreatitis was diagnosed in 4 (9.8%) dogs and gastrointestinal disease in 3 (7.3%) dogs (small bowel diarrhea, hematochezia and coccidiosis, and regurgitation in 1 dog each). Liver disease was diagnosed in 3 (7.3%) dogs including a dog with an extrahepatic portosystemic shunt, a dog with suspected aflatoxin exposure, and a dog with an undefined hepatopathy. Cardiac diseases were diagnosed in 3 (7.3%) older dogs (age range, 8-12 years) and included mitral valve endocarditis with aortic valve stenosis in a Labrador Retriever, mitral valve regurgitation in a mixed breed, and atrioventricular (AV) endocarditis with supraventricular arrhythmia in an American Cocker Spaniel. Immune-mediated thrombocytopenia (ITP), hyperadrenocorticism (HAC), and uveitis each were diagnosed in 2 (4.9%) dogs. Lymphocytosis was diagnosed in

2 (4.9%) dogs, including 1 dog with CLL based on a high lymphocyte count (44% lymphocytes) and high CD79b staining by flow cytometry and 1 dog with 39% abnormal lymphocytes that was diagnosed with CLL 7 months later. Two dogs were not diagnosed with a disease, including 1 dog with no clinical signs and 1 dog that presented with generalized pain.

Ehrlichiosis was diagnosed during the initial diagnostic evaluation in 28 of 41 (68.3%) dogs in our study, all of which were treated with doxycycline, except 1 dog that was euthanized. Based on suspicion of an infectious etiology, doxycycline treatment was administered to 7 of 13 (53.8%) dogs not initially diagnosed with ehrlichiosis or was started approximately 1 month after the initial assessment in 4 of the 13 (30.8%) dogs. There was no record of doxycycline treatment in 2 of the 13 (15.4%) dogs.

3.4 | Physical examination findings

Physical examination findings varied and were nonspecific (Figure 2). The most frequently recorded physical examination findings were limb

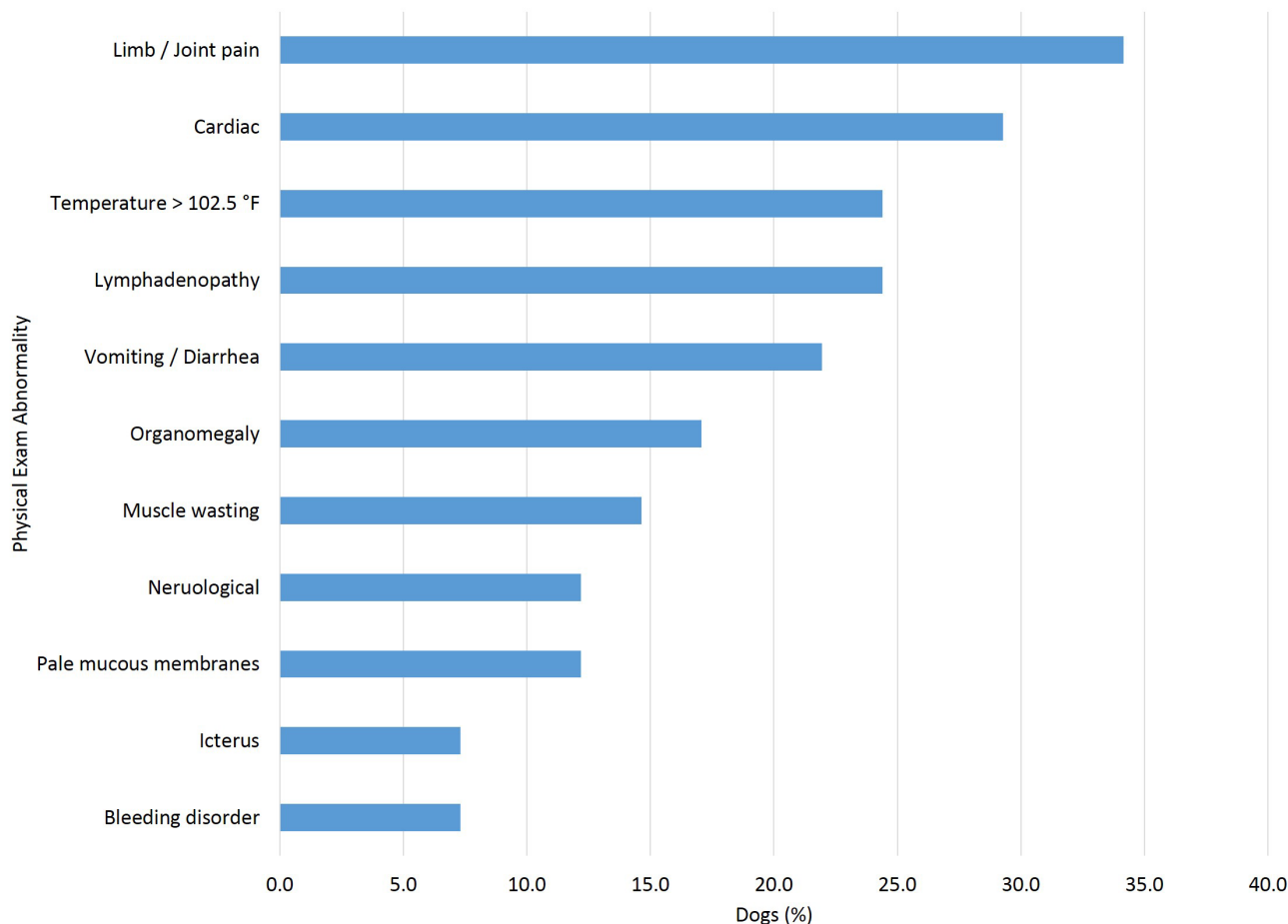


FIGURE 2 Predominant physical examination findings in 41 *Ehrlichia ewingii* PCR-positive dogs. Abnormality proportions are organized from highest to lowest

or joint pain (14/41; 34.1%), cardiac abnormalities (12/41; 29.3%), fever (10/41; 24.4%), lymphadenopathy (10/41; 24.4%), vomiting or diarrhea or both (9/41; 22%), and organomegaly (7/41; 17.1%). Of the 14 dogs with limb or joint pain, 11 (84.6%) had polyarthralgia, which included crepitus, decreased range of motion, painful palpation of joints, swelling or effusion, or both, of multiple joints; 4 dogs had joint effusion and 1 dog had neutrophils with *Ehrlichia morula* identified by joint cytology. Of the remaining dogs with limb or joint pain, 1 was described as having a non-weight bearing, shifting pelvic limb lameness, 1 was referred for evaluation of proteinuria and preputial discharge 2 weeks after orthopedic surgery, and 1 dog had temporomandibular joint pain. Of the 12 dogs with auscultated cardiac abnormalities, 1 (8.3%) had premature beats and pulse deficits and 11 (91.7%) dogs had heart murmurs, graded between II and IV/VI. Four dogs with heart murmurs were severely anemic (PCV range, 12%-16%). Of the 10 dogs with peripheral lymphadenopathy, popliteal lymph nodes (7/10; 70%) most often were enlarged. Five dogs with enlarged popliteal lymph nodes had concurrent lameness, joint effusion, or crepitus in ≥ 1 pelvic limbs. Other enlarged lymph nodes included the superficial cervical, mandibular, prescapular, and retropharyngeal lymph nodes. Of the 7 dogs with organomegaly, 3 (42.9%) had splenomegaly and 4 (57.1%) had hepatomegaly or non-specified cranial organomegaly. Bleeding was noted in 3 of 41 (7.3%) dogs, including 2 dogs with epistaxis and 1 dog with hyphema and

petechia. One *E. ewingii* PCR-positive dog had no clinical abnormalities at a wellness examination.

3.5 | Laboratory abnormalities

Complete blood counts were available for 37 of 41 (90.2%) dogs (Table 2). Evaluation of lymphocyte morphology, as assessed by clinical pathology technologists (Clinical Pathology department at the North Carolina State University – College of Veterinary Medicine–Veterinary Hospital, Raleigh, NC) for 36 dogs, identified abnormal lymphocytes (22/36; 61.1%) as the most prominent hematologic abnormality. Normal lymphocytes were within or below the reference intervals for all dogs except 1 (2.7%) dog with CLL but without abnormal lymphocyte appearance. Relative numbers of abnormal lymphocytes ranged from 1% to 39%, (mean, 7.4%). Immune-mediated thrombocytopenia secondary to ehrlichiosis was diagnosed in a dog with 38% abnormal lymphocytes. A dog with 39% abnormal lymphocytes had expansion of CD8+ T cells with PCR for antigen receptor rearrangements (PARR) supporting clonal T-cell expansion consistent with T-cell malignancy or chronic antigenic stimulation caused by *Ehrlichia* infection. This dog was treated with doxycycline (5 mg/kg, q12h, PO for 1 month), but CLL was diagnosed 7 months after initial presentation because of a progressive increase in abnormal lymphocytes; the dog was not retested for *E. ewingii* clearance after

TABLE 2 Predominant CBC abnormalities in 37 *Ehrlichia ewingii* PCR-positive dogs

CBC abnormality	Dogs with abnormality (%)	Range of abnormal values	Median	Mean	SD	Reference intervals (RIs) ^a
Abnormal lymphocytes ^b	22/36 (61.1)	0.103-5.441	0.56	0.803	1.11	Abnormal lymphs ($\times 10^3/\mu\text{L}$) No RI
Neutrophilia	21/37 (56.8)	9.4-48.1	14.8	17.6	9.55	Neutrophils ($\times 10^3/\mu\text{L}$) 2.841-9.112 2.06-10.6
Leukocytosis	20/37 (54.1)	12.9-51.2	18.3	22.3	9.62	WBC ($\times 10^3/\mu\text{L}$) 4.39-11.61 4.0-15.5
Anemia	18/37 (48.6)	12-38	30	28.3	9.05	
Non-regenerative	11/37 (29.7)	28-38	33	33.4	3.9	39-58 (PCV, %)
Regenerative	7/37 (18.9)	12-38	16.5	20.4	9.39	36-60 (HCT, %)
Spherocytes	6/37 (16.2)	NA	NA	NA	NA	
Monocytosis	17/37 (45.9)	0.99-6.45	1.88	2.19	1.54	Monocytes ($\times 10^3/\mu\text{L}$) 0.75-0.85 0-0.84
Hyperproteinemia ^c	16/36 (44.4)	7.6-9.3	8.2	8.1	0.73	PP (g/dL) 6.1-7.5
Thrombocytopenia	16/37 (43.2)	0.5-189	159.5	124.7	65.72	Platelets ($\times 10^3/\mu\text{L}$) 190-468 170-400
Band neutrophils	16/37 (43.2)	0.058-2.9	0.485	0.664	0.734	Bands ($\times 10^3/\mu\text{L}$) RI 0-0.3
Lymphopenia	14/37 (37.8)	0.036-0.571	0.31	0.31	0.157	Lymphs ($\times 10^3/\mu\text{L}$) 0.594-3.305 0.690-4.500
Morulae in neutrophils	6/37 (16.2)	NA	NA	NA	NA	No RI
Thrombocytosis	3/37 (8.1)	466-713	494	558	135.25	Platelets ($\times 10^3/\mu\text{L}$) 190-468 170-400
Leukopenia	2/37 (5.4)	3.37, 3.61	3.49	3.49	0.169	WBC ($\times 10^3/\mu\text{L}$) 4.39-11.61 4.0-15.5
Neutropenia	2/37 (5.4)	2.022, 2.1	2.061	2.061	0.055	Neutrophils ($\times 10^3/\mu\text{L}$) 2.841-9.112 2.06-10.6
Eosinophilia	1/37 (2.7)	1.329	NA	NA	NA	Eosinophils ($\times 10^3/\mu\text{L}$) 0.03-1.264 0-1.2
Lymphocytosis	1/37 (2.7)	11.71	NA	NA	NA	Lymphs ($\times 10^3/\mu\text{L}$) 0.594-3.305 0.690-4.500

Abnormality proportions are organized from highest to lowest.

Abbreviation: NA, not applicable.

^a RIs represent 2 different CBC analyses: Clinical Pathology Department at the North Carolina State University Veterinary Hospital (n = 36 dogs) and Antech Diagnostics (n = 1 dog).

^b Abnormal lymphocytes were determined for samples analyzed through the Clinical Pathology Department at the North Carolina State University Veterinary Hospital (n = 36 dogs).

^c Plasma protein was not a component of 1 canine CBC analyses.

doxycycline treatment. Neutrophilia was detected in 21 of 37 (56.8%) dogs, and band neutrophils were reported in 16 of 37 (43.1%) dogs. The NLR was increased (expected value <5) in 27 of 37 (72.9%) dogs, with a mean of 20.3 (range, 5-90.8). Neutrophils containing

cytoplasmic morula were reported in 6 of 37 (16.2%) dogs. Anemia was reported in 18 of 37 (48.6%) dogs, with 7 of 18 (38.9%) dogs characterized as regenerative on the basis of reticulocytosis. Monocytosis was detected in 17 of 37 (45.9%) dogs, and thrombocytopenia

TABLE 3 Predominant serum biochemical abnormalities in 35 *Ehrlichia ewingii* PCR-positive dogs

Biochemistry abnormality	Dogs with abnormality (%)	Range of abnormal values	Median	Mean	SD	Reference intervals ^a
Increased ALP	20/35 (57.1)	146-1114	256.5	358.5	275.02	IU/L 16-140 5-131 20-150
Increased ALT	14/35 (40)	55-8520	103.5	792.9	2240.08	IU/L 12-54 12-118 10-118
Increased SDMA ^b	11/34 (32.4)	38-74	25	38	23.62	µg/dL 0-14
Increased BUN	11/35 (31.4)	29-233	54	72.9	62.69	mg/dL 6-26 6-31 7-25
Hypoalbuminemia	11/35 (31.4)	2.2-2.9	2.6	2.64	0.23	g/dL 3-3.9 2.7-4.4 2.5-4.4
Increased CK	10/32 (31.3)	243-1690	566	601	429.11	IU/L 43-234 59-895
Increased amylase	10/35 (28.6)	1206-18 270	1602	3350	5254.32	IU/L 236-1337 290-1125 200-1200
Increased lipase	8/32 (25)	151-18 240	1055	3298	6135.56	IU/L 12-147 77-695
Hypercholesterolemia	8/32 (25)	374-472	390.5	405	37.79	mg/dL 124-344 92-324
Hyperbilirubinemia	8/35 (22.9)	0.3-5	0.9	1.41	1.61	mg/dL 0.0-1.2 0.1-0.3 0.1-0.6
Low A/G	8/35 (22.9)	0.59-0.87	0.71	0.72	0.27	Ratio 0.90-1.8 0.80-2.0
Increased GGT	6/35 (18.8)	7-10	8.5	8.33	1.21	IU/L 0-6 1-12
Increased creatinine	6/35 (17.1)	1.9-13.1	4.85	5.58	4.01	mg/dL 0.7-1.5 0.5-1.6 0.3-1.4
Hypochloremia	5/32 (15.6)	101-107	102	103.4	2.88	mmol/L 108-122 102-120
Hyperphosphatemia	5/35 (14.3)	6-18.1	9.2	10.22	4.81	mg/dL 2.5-5.6 2.9-6.6 2.5-6.0

(Continues)

TABLE 3 (Continued)

Biochemistry abnormality	Dogs with abnormality (%)	Range of abnormal values	Median	Mean	SD	Reference intervals ^a
Hyperglycemia	5/35 (14.3)	111-288	133	168	70.97	mg/dL 70-131 70-138 60-110
Hypokalemia	5/35 (14.3)	3.2-3.9	3.5	3.56	0.26	mmol/L 4-5.3 3.7-5.8 3.6-5.5
Hyperglobulinemia	4/35 (11.4)	3.9-4.6	4.3	4.3	0.29	g/dL 1.7-3.8 1.6-3.6 2.3-5.2

Abnormality proportions are organized from highest to lowest.

Abbreviations: A/G, albumin to globulin ratio; ALP, alkaline phosphatase; ALT, alanine transaminase; BUN, blood urea nitrogen; CK, creatine kinase; GGT, gamma-glutamyl transferase; SDMA, symmetric dimethylarginine.

^a Reference intervals represent 3 different serum biochemical analyses: Clinical Pathology Department at the North Carolina State University Veterinary Hospital (n = 31 dogs); Abaxis after hours at the North Carolina State University Veterinary Hospital (n = 3 dogs); and Antech Diagnostics (n = 1 dog).

^b SDMA test was performed retrospectively on 34 dogs, by IDEXX Laboratories, Inc.

was detected in 16 of 37 (43.2%) dogs. The 2 dogs diagnosed with ITP had the lowest platelet counts at 0.5 and $30 \times 10^3 \mu\text{L}$.

Serum biochemistry panels were available for 35 of 41 (85.4%) dogs (Table 3), including 3 dogs tested by after-hours panels (Clinical Pathology department at the North Carolina State University – College of Veterinary Medicine-Veterinary Hospital, Raleigh, NC) with limited biochemical tests. Increased alkaline phosphatase (ALP; 20/35; 57.1%) and alanine transaminase (ALT; 14/35; 40%) activities were the most prominent abnormalities. Of the 20 dogs with increased liver enzyme activities, 2 had received glucocorticoids before presentation, 3 others were diagnosed with hepatopathies, and 1 dog was diagnosed with HAC. Another dog diagnosed with HAC did not have concurrent serum biochemistry data. Increased blood urea nitrogen (BUN) (11/35; 31.4%) concentration and hypoalbuminemia (11/35; 31.1%) also were frequently detected. Stored serum for retrospective SDMA testing was available for 34 of 41 (82.9%) dogs. Increased SDMA concentrations of $>14 \mu\text{g/dL}$ were identified in 11 of 34 (32.4%) dogs (range, 15-74 $\mu\text{g/dL}$; reference interval, 0-14 $\mu\text{g/dL}$).

Urinalyses were available for 27 of 41 (65.9%) dogs and bacterial urine culture results for 20 of 27 (74%) dogs. Proteinuria was detected in 20 of 27 (74%) dogs (dipstick protein concentrations $\geq 30 \text{ mg/dL}$ or $\geq 15 \text{ mg/dL}$ in the presence of dilute urine), of which 17 of the 20 (85%) dogs had microalbuminemia (sulfosalicylic acid concentrations $\geq 15 \text{ mg/dL}$) and 16 of the 20 (80%) dogs had an inactive sediment (defined as $<5 \text{ WBC/hpf}$, $<20 \text{ RBC/hpf}$, and no visible bacteria). Of the proteinuric dogs with inactive sediment, 10 of 16 (62.5%) had blood pressure measured, of which 3 of the 10 (30%) dogs were hypertensive, with systolic blood pressures between 190 and 250 mm Hg. Urine protein : creatinine ratios (UP/C) were measured in 10 dogs, of which 2 dogs had active sediments. The UP/C ratios were >0.5 , (range, 0.7-9.75) in the 8 dogs with inactive sediments, with a UP/C >0.5 indicative of proteinuria in dogs.²² Only 1 of 20 (5%) urine culture specimens grew bacteria (*Staphylococcus pseudointermedius*).

4 | DISCUSSION

Including dogs with renal disease, proteinuria was detected in a high number of *E. ewingii* PCR-positive dogs (20/27; 74%) in our study, most of which had inactive urine sediments. When compared to previous studies, the high proportion of *E. ewingii*-infected dogs with proteinuria (74%) or kidney disease (17.1%) represented the most notable clinicopathological finding in our retrospective descriptive study. To our knowledge, only 1 natural infection CGE study reported proteinuria in dogs with active sediments (2/5; 40%).¹⁶ One study showed that 79% of dogs with CME were proteinuric.²³ In our study, PLN and CKD were diagnosed in 1 and 6 dogs, respectively, with disease onset ranging from 1 month to 2 years before documentation of an *E. ewingii* PCR-positive result; 4 of those dogs presented with A-CKD. The chronic kidney disease in these dogs is likely multifactorial. As often occurs, the etiology of kidney disease was not definitively determined for the dogs in our study. The UP/C ratios, available for 6 of 7 dogs with renal disease, indicated glomerular pathology such as glomerulonephritis. Leptospirosis results were negative for the 4 dogs tested. In addition to renal causes of proteinuria, prerenal conditions may contribute to proteinuria. Most of the proteinuric dogs in our study with an inactive sediment and a blood pressure measurement were normotensive (70%). Three other proteinuric A-CKD or CKD dogs were diagnosed with concurrent pancreatitis, which could have contributed, in part, to the proteinuria. *Ehrlichia* is considered a potential etiological agent for glomerular disease that may induce glomerular injury secondary to immune complex deposition.²⁴⁻²⁶ Membranoproliferative glomerulonephritis was reported in a human infected with *E. chaffeensis*.²⁶ Dogs experimentally infected with *E. canis* developed transient proteinuria, increased UP/C, and hypoalbuminemia with glomerular deposition of immune complexes.²⁷ Morphological changes in renal biopsy specimens from the dogs experimentally infected with *E. canis* documented glomerular proteinuria (IgM and IgG deposits in the glomeruli) and minimal change

glomerulopathy.²⁸ Seven hypoalbuminemic dogs in our study had concurrent proteinuria. Necropsy of 1 proteinuric, non-azotemic dog in our study identified lymphoplasmacytic interstitial nephritis and glomerular amyloidosis.

Concentrations of SDMA, a recently introduced biomarker for early renal dysfunction, were increased in all 7 dogs diagnosed with renal disease, including 6 azotemic dogs and 1 non-azotemic dog with PLN. Four other non-azotemic dogs had increased SDMA concentrations, suggestive of potential early kidney dysfunction, 2 of which had urinalyses performed that indicated that 1 dog was proteinuric with an inactive sediment. Seven other dogs with proteinuria and inactive sediment had normal SDMA concentrations, supporting the absence of detectable kidney dysfunction despite proteinuria.

The 7 dogs diagnosed with renal disease in our study ranged in age from 9-12 years, thus, *E. ewingii* infection may or may not have played a contributing role in renal pathology. Chronic infection with *E. ewingii*, coupled with immune senescence in older dogs, might contribute to progressive renal injury and PLN. The high percentage of dogs with proteinuria in our study warrants further investigation of chronic *E. ewingii* infections in the progression of kidney disease. Furthermore, clinicians should consider testing for proteinuria in *E. ewingii* PCR positive or *Ehrlichia* SNAP[®] enzyme-linked immunosorbent assay seroreactive dogs.

Immune-mediated disease (6 IMHA and 2 ITP dogs) was diagnosed in 8 of 41 (19.5%) *E. ewingii*-infected dogs. All 6 IMHA dogs in our study had regenerative anemia, spherocytes, or agglutination or some combination of these, and all dogs received extensive diagnostic evaluation to identify secondary causes of IMHA. All but 1 dog had historical tick exposure recorded in the medical record. Based on the positive *Ehrlichia* PCR or serology results, 5 of 6 dogs were diagnosed with secondary IMHA, whereas 1 dog was diagnosed with primary IMHA. All IMHA dogs, regardless of their CVBP diagnostic results, were treated with doxycycline, immunosuppressive drugs, and supportive care and were discharged from the hospital. Of the 6 IMHA dogs, 2 were lost to follow-up; 1 was no longer anemic 3 months after discharge; 1 concurrently diagnosed with A-CKD developed progressive renal failure, but did not experience an IMHA relapse; and, 2 dogs relapsed, developing IMHA again at 7 months and 2 years after discharge. Interestingly, 1 dog was PCR positive for *E. ewingii* again at the time of relapse despite having been treated with doxycycline and thus likely failed treatment or was reinfected. Of the 2 ITP dogs in our study, 1 treated with doxycycline was lost to follow-up and the 2nd dog was treated with prednisone, azathioprine, and doxycycline but was euthanized 1 month later after developing severe anemia and severe neutrophilia. A bone marrow cytology performed before ITP diagnosis for this dog was normal, suggesting that ITP was not because of impaired platelet production. Historically, studies relating to CVBP, IMHA, ITP, or some combination of these have addressed case reports or case series, in contrast to longitudinal treatment and follow-up investigations. In a group of 56 dogs from the southeastern United States diagnosed with IMHA, ITP, or both, 30% were exposed to or infected with a CVBP, and the most prevalent pathogen detected by PCR was *E. ewingii* (7%).²⁹ *E. ewingii* was reported in 3 IMHA dogs in southern California.³¹ Historically, a comprehensive diagnostic approach to rule in or rule out infection with CVBP in dogs

with IMHA or ITP has not been consistently pursued.^{30,31} Clinicians are more likely to test for *Babesia* infections, because hemolytic anemia often is linked with this particular CVBP.^{32,33} Based on previous reports and our study, *E. ewingii* may play a more important role in the pathogenesis of IMHA and ITP than previously suspected. Also, recent evidence supports CVBP misdiagnosis in dogs that were subsequently PCR positive after immunosuppressive drug treatment.³¹ Immunosuppressive treatment in dogs with occult intravascular CVBP infections may in part contribute to the high mortality rate in dogs with IMHA.

Physical examination findings were nonspecific, involved multiple organ systems, and varied substantially among individual dogs. Consistent with previous reports of *E. ewingii*-infected dogs, limb or joint pain was the most common physical examination finding in our study.^{14,19,20} Cardiac abnormalities were the 2nd most common physical examination finding, with 4 of 12 (33.3%) dogs possibly attributed to anemic heart murmurs. Breeds with dispositions for cardiac disease accounted for 8 of 12 (66.7%) cases in our study and included a Bullmastiff with a history of pulmonic stenosis, a Miniature Schnauzer, a Pomeranian, a Shih Tzu, a Jack Russell Terrier, a Labrador Retriever, a Collie, and an American Cocker Spaniel.³⁴ Furthermore, the average age of dogs with cardiac signs in our study was 7.8 years, increasing the risk of age-related cardiac disease. A few reports, however, have describe a potential role for *E. canis* as a cause of cardiac disease in dogs with increased cardiac troponin 1 in dogs naturally infected with *E. canis*.^{35,36} Gastrointestinal signs before doxycycline administration also were commonly reported among dogs in our study. Vomiting and diarrhea have not been historically attributed to ehrlichiosis in dogs, but in human medicine, gastrointestinal signs frequently are reported as a component of *E. chaffeensis* or *E. ewingii* infections.^{3,37,38} Fever was common relative to other physical examination findings in our study, but more dogs were afebrile (75.6%), which may indicate a chronic phase of infection. One *E. ewingii* experimental infection study compared clinical signs between a group of naive dogs and a group of previously *E. ewingii*-infected dogs.¹⁷ Dogs that had previously been infected with and treated for *E. ewingii* remained afebrile, whereas the naive dogs developed intermittent fever. The physical examination findings recorded for dogs in our study support previous findings of musculoskeletal pain and fever in *E. ewingii*-infected dogs, but clinicians should consider less common presentations in afebrile dogs with ehrlichemia, including cardiac abnormalities, gastrointestinal signs, and lymphadenopathy.

Predominant hematologic abnormalities identified in this group of *E. ewingii*-infected dogs included abnormal circulating lymphocytes, neutrophilia, and increased liver enzyme activities. Although nonspecific, abnormal lymphocytes were the most common CBC abnormality, reported in 61.1% of these *E. ewingii*-infected dogs. Abnormal lymphocytes, defined as small to intermediate in size with increased amounts of pale cytoplasm (without a blast appearance), have similar diagnostic relevance as reactive lymphocytes. Thus, the increase in abnormal lymphocytes likely reflects increased antigenic stimulation. Two other studies reported reactive lymphocytes in 35%-38% of *E. ewingii*-infected dogs.^{14,19} Nine (24.3%) of the dogs in our study had reactive lymphocytes, but all were within the reference intervals. Mild to moderate neutrophilia was identified in 56.8% of the dogs in our study, supporting the presence of a mild inflammatory response.

Neutrophilia has been reported in 30%–33% of dogs naturally infected with *E. ewingii*.^{14,16} Less than half of the *E. ewingii*-infected dogs (43.2%) in our study were thrombocytopenic. Thrombocytopenia was more frequent in other reports of *E. ewingii*-infected dogs, ranging from 83% to 100% of affected dogs, potentially because of preferential clinician selection of thrombocytopenic dogs for testing.^{14,16,19,20} Variation could represent selection criteria for testing, virulence differences among *E. ewingii* strains, pathophysiological differences in host response during various stages of infection (acute, subclinical, and chronic), or decreased clinical disease because of a previous *E. ewingii* infection. Two dogs experimentally infected with *E. ewingii*, treated with doxycycline, and then *E. ewingii* challenged did not develop thrombocytopenia, unlike naive dogs infected with *E. ewingii*.¹⁷ Mild increases in ALT and ALP activities were the most common serum biochemical abnormalities identified in our study. Increased liver enzyme activities also have been reported for dogs infected with *E. ewingii*, *E. canis*, and *Anaplasma phagocytophilum*.^{14,39–42} Hepatic involvement with mild increases in liver enzyme activities is a common finding in people with ehrlichiosis.³⁷ In addition to more historically associated hematological abnormalities such as thrombocytopenia, veterinarians should consider screening for CGE when abnormal or reactive lymphocytes, neutrophilia, and increased ALT or ALP activities are observed in their canine patients.

The NLR was increased in a large portion of dogs (73%) in our study. Increased NLR is an indicator of systemic inflammation and is associated with increased circulating cytokines.⁴³ Neutrophil-to-lymphocyte ratio has been used as a prognostic factor in both humans and dogs with malignancies.^{44–49} Evaluating NLR can compensate for fluctuations between individual neutrophil and lymphocyte counts. One study measured NLR in dogs infected with *B. canis* and made comparisons between dogs with uncomplicated babesiosis (dogs with fever or anemia) and complicated babesiosis (dogs that developed acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, IMHA, acute respiratory distress syndrome, or shock).^{50,51} The NLRs were significantly higher in dogs with complicated as compared to uncomplicated babesiosis. Although increased neutrophil and decreased lymphocyte numbers occur in association with stress leukograms in dogs, the NLR ratios reported in stressed dogs were lower (mean, 7.43 ± 4.2) than the those reported in our study (mean, 20.3 ± 6.9).²¹ Potentially, NLR could serve as a diagnostic indicator warranting CVBP testing.

Based on recent publications, asymptomatic dogs may serve as reservoir hosts for *E. ewingii*.^{18,52} Dogs exposed to *E. ewingii* can become persistently infected and remain asymptomatic for years, with a subset developing clinical disease. Reported differences in clinical presentation in dogs infected with *E. ewingii* could be because of the age, sex, and health of the dog; immune status, which may include a state of compromise or premunition; duration of infection; and strain variances or coinfections with other CVBP. Vector-borne pathogens have coevolved with their respective reservoir hosts to induce persistent infections and invoke immune evasive mechanisms that protect the pathogen from elimination. Factors that induce immune dysregulation, chronic inflammation, and pathophysiological decompensation are likely complex and may vary among individual patients.^{53–56} Determining the extent to which persistent CVBP infections contribute to chronic diseases after natural infection would require sequential testing and long-term studies.

In 1 study, dogs experimentally infected with *E. canis* and *E. chaffeensis* remained persistently infected for 42 days before euthanasia.⁵⁷ Both pathogens were detected by PCR in lung, liver, spleen, and lymph nodes. Histopathological analysis identified microgranulomas and perivascular infiltrates of macrophages and lymphocytes in lungs and liver, and lymphoid hyperplasia in the spleen. Based on the physical examination findings (lymphadenopathy and organomegaly), hematological findings, and biochemical abnormalities, the *E. ewingii*-infected dogs in our study had clear clinical, hematological, and biochemical indications of multiorgan system pathology. Determining the potential biological price that a dog might pay for longstanding infection with any vector-borne, bloodstream-adapted organism is difficult to study in the clinical setting, because our data often are limited to a single point in time once clinical illness develops. Based on our results, persistent *E. ewingii* infection may contribute to comorbidities or function as a precipitating factor in a spectrum of clinical syndromes in dogs.

Vector-borne coinfections are not uncommon in dogs with vector exposures.⁵⁸ Because coinfections often produce atypical clinical presentations and increase illness severity, we attempted to systematically exclude coinfections with known CVBP by PCR testing. Diagnostically, PCR is a sensitive molecular-based assay for detecting CVBP, but a negative PCR result cannot definitively rule out an infection. The vector-borne pathogen load can vary depending on the organism and stage (acute versus chronic) of infection. In an effort to assess study results in the context of *E. ewingii*-PCR positivity, all the dogs in our study were blood PCR negative for other CVBP and most were concurrently seronegative. We did not eliminate dogs that were seroreactive to other CVBP that may only signify prior exposures. The larger number of dogs in our study that were *R. rickettsii* seroreactive likely indicates antibodies to spotted-fever group rickettsia (SFGR), which are prevalent among dogs in the southeastern United States.⁵⁹ This high seroprevalence is not reflective of a prior or current case of Rocky Mountain spotted fever, but rather exposure to less pathogenic, more prevalent SFGR.^{60,61}

Our retrospective study had several limitations. The dogs evaluated in our study were selected from a population of dogs from which clinicians submitted samples for CVBP diagnostic testing. Thus, there was a selection bias toward clinicopathologic abnormalities expected in dogs with CVBP. The clinical signs reported may have been over-represented because of self-selection. All but 1 dog was evaluated at a veterinary referral hospital, creating a bias for dogs with severe disease. There was a lack of complete clinical information for all dogs, including limited history and follow-up evaluations. Ours was a descriptive study and not designed to prove causation of the diseases or clinicopathologic abnormalities observed in the *E. ewingii*-infected dogs. Although efforts were made to rule out coinfections with other CVBP, coinfections still could have been present in dogs that were falsely PCR negative or that may have been infected with an unknown or untested CVBP.

5 | CONCLUSION

Our study described historical, clinical, hematological, and biochemical abnormalities reported in dogs naturally infected with *E. ewingii*. We

identified a relatively high proportion of dogs diagnosed with renal disease and IMHA, medical conditions not previously associated with *E. ewingii* infection in dogs. Frequently documented clinicopathological abnormalities included proteinuria, increased numbers of abnormal lymphocytes, neutrophilia, increased NLR, and increased liver enzyme activities. Future studies are needed to determine if *E. ewingii* contributes to comorbidities or if a more diverse spectrum of clinical syndromes occurs in persistently infected dogs than is currently recognized.

ACKNOWLEDGMENTS

We thank the numerous clinicians and diagnostic personnel at the NC State-VH for generating the case-based data summarized in our study. We also thank the personnel of the North Carolina State University, College of Veterinary Medicine, Vector-borne Disease Diagnostic Laboratory for diagnostic testing and helpful comments regarding this article. This work was completed at North Carolina State University, College of Veterinary Medicine, Vector-borne Disease Diagnostic Laboratory. The results from our study were presented at the 2017 ACVIM Forum, National Harbor, Maryland.

CONFLICT OF INTEREST DECLARATION

B. Qurollo is a research assistant professor at NC State-CVM, co-director of the NC State-CVM-VBDDL and a vector borne disease researcher. IDEXX Laboratories, Inc funds a portion of her salary. J. Buch, R. Chandrashekar, and M. J. Beall work for IDEXX Laboratories, Inc. E. B. Breitschwerdt co-directs the VBDDL and the Intracellular Pathogens Research Laboratory at NC State, is chief scientific officer at Galaxy Diagnostics and a paid consultant for IDEXX Laboratories, Inc.

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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How to cite this article: Qurollo BA, Buch J, Chandrashekar R, et al. Clinicopathological findings in 41 dogs (2008-2018) naturally infected with *Ehrlichia ewingii*. *J Vet Intern Med.* 2019;33:618-629. <https://doi.org/10.1111/jvim.15354>