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Differences in clinicopathologic variables between *Borrelia* C6 antigen seroreactive and *Borrelia* C6 seronegative glomerulopathy in dogs

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Abstract

Background: Rapidly progressive glomerulonephritis has been described in dogs that seroreact to *Borrelia burgdorferi*, but no studies have compared clinicopathologic differences in Lyme-seroreactive dogs with protein-losing nephropathy (PLN) versus dogs with *Borrelia*-seronegative PLN.

Hypothesis/Objectives: Dogs with *Borrelia* C6 antigen-seroreactive PLN have distinct clinicopathologic findings when compared to dogs with *Borrelia* seronegative PLN.

Animals: Forty dogs with PLN and *Borrelia* C6 antigen seroreactivity and 78 C6-seronegative temporally matched dogs with PLN.

Methods: Retrospective prevalence case-control study. Clinical information was retrieved from records of dogs examined at the University of California, Davis, Veterinary Medical Teaching Hospital. Histopathologic findings in renal tissue procured by biopsy or necropsy of dogs with PLN were reviewed.

Results: Retrievers and retriever mixes were overrepresented in seroreactive dogs ($P < .001$). Seroreactive dogs were more likely to have thrombocytopenia ($P < .001$), azotemia ($P = .002$), hyperphosphatemia ($P < .001$), anemia ($P < .001$), and neutrophilia ($P = .003$). Hematuria, glucosuria, and pyuria despite negative urine culture were more likely in seroreactive dogs (all $P \leq .002$). Histopathologic findings were consistent with immune-complex glomerulonephritis in 16 of 16 case dogs and 7 of 23 control dogs ($P = 006$). Prevalence of polyarthritis was not different between groups ($P = .17$).

Conclusions and Clinical Importance: C6 seroreactivity in dogs with PLN is associated with a clinicopathologically distinct syndrome when compared with other types of PLN. Early recognition of this syndrome has the potential to improve outcomes through specific aggressive and early treatment.

KEYWORDS

glomerulonephritis, Lyme disease, PLN, polyarthritis, pyuria, thrombocytopenia

Abbreviations: CI, confidence interval; HLA, histocompatibility leukocyte antigen; ICGN, immune complex glomerulonephritis; MCV, mean corpuscular volume; MGN, membranous glomerulonephritis; PLN, protein-losing nephropathy; UPCR, urine protein:creatinine ratio; VMTH, Veterinary Medical Teaching Hospital.

1 | INTRODUCTION

The diagnosis of Lyme borreliosis remains challenging for veterinary practitioners presented with dogs with polyarthritis or protein-losing nephropathy (PLN). Similarly, Lyme disease is the most commonly reported tick-borne disease in humans in the United States,[1] but proving a correlation between positive serologic testing and Lyme disease is difficult. The distribution of Lyme disease in the United States is largely determined by its 2 primary tick vectors, *Ixodes scapularis* and *Ixodes pacificus*, and is concentrated in the northeast and upper midwest, and along the Pacific coast. In these areas, up to 40% of healthy dogs may test positive for antibodies to *Borrelia burgdorferi*,[2-4] but only a small percentage (<10%) of infected dogs are believed to develop clinical signs of disease.[5,6] Because organisms are present in very low numbers in connective tissue and are difficult to detect using culture or molecular techniques, diagnosis of acute Lyme borreliosis relies on a combination of characteristic clinical signs (polyarthritis, fever, inappetence), exclusion of other systemic diseases, and seroreactivity to *Borrelia* antigens. The *B. burgdorferi* C6 peptide antigen is a portion of the spirochete VlsE surface protein and has been a popular target for serologic assays, because it is only expressed in the host during active infection.[7]

The extended time course of the disease makes it even more difficult for practitioners to associate chronic disorders with *Borrelia* infection. Rheumatologic, neurologic, and cardiac complications have been recognized in humans.[8-10] Correlations with fibromyalgia, memory impairment, and chronic fatigue also have been proposed in the human medical literature, but the diagnosis of "chronic Lyme disease" remains controversial.[9,11,12] All these conditions are poorly documented in dogs with borreliosis.[13-15] Conversely, a syndrome of severe PLN ("Lyme nephritis") is believed to occur in 5%-10% of affected dogs, whereas reports of similar renal complications in human patients are rare.[16,17]

Lyme nephritis is a syndrome of rapidly progressive membranoproliferative glomerulonephritis in *Borrelia*-seroreactive dogs that was first described in 1997.[18] Dogs with Lyme nephritis were significantly younger than dogs with other types of glomerulonephritis, and Golden Retrievers and Labrador Retrievers were overrepresented. Clinicopathologic findings were not described in that study, and to our knowledge, no other studies that compare findings in dogs with suspected Lyme nephritis to findings in dogs with other causes of PLN have been undertaken to date.

We have observed that thrombocytopenia and concurrent polyarthritis are often present in dogs with *Borrelia* C6 antigen-positive PLN but appear to be uncommon in dogs with other types of PLN. Therefore, our goal was to determine whether dogs in northern California with PLN that were seroreactive to the *Borrelia* C6 antigen had distinct clinicopathologic abnormalities that distinguished them from dogs with PLN that were seronegative for the *Borrelia* C6 antigen. We hypothesized that platelet counts would be lower in seroreactive dogs as compared to seronegative dogs, and that the prevalence of polyarthritis would be higher in seroreactive dogs. With this study, we aimed to strengthen the existing evidence that Lyme nephritis is a unique clinical syndrome and to identify clinicopathologic abnormalities that might assist in the early recognition of the disease.

2 | MATERIALS AND METHODS

This study was a retrospective prevalence case-control study. All dogs evaluated at the Veterinary Medical Teaching Hospital (VMTH) at the University of California, Davis, between January 2002 and December 2015, were eligible for inclusion. Electronic medical records were searched for dogs evaluated for PLN that also had vector-borne disease serology performed that included a C6 peptide antibody test (Quantitative C6, SNAP 3DX, SNAP 4DX, and SNAP 4DX Plus, IDEXX Laboratories, Portland, Maine) within 1 month of first being evaluated. For every dog identified with PLN seroreactive for *Borrelia*, 2 temporally associated seronegative control dogs evaluated for PLN were selected for comparison—the dogs seen immediately before and after the seroreactive case (ie, 2 controls for each case). Additional inclusion criteria for all case and control dogs were a urine protein:creatinine ratio (UPCR) >5 or histologically confirmed glomerulonephritis. Dogs were excluded if results of a UPCR performed within 1 month of initial evaluation were not available. Patient age, breed, sex, body weight, rectal temperature at the time of evaluation, clinical signs, and date of onset of illness were recorded. Where performed, results of blood pressure evaluation were recorded and classified as hypertensive (systolic pressure >160 mm Hg) or normotensive. Results of a CBC, serum biochemistry panel, urinalysis, aerobic bacterial urine culture, and serologic testing for other vector-borne diseases, performed within 1 month of initial evaluation, either at the VMTH or at a commercial veterinary diagnostic laboratory, were collected. When the same tests had been performed multiple times, the test performed closest to the time of initial examination for PLN at the VMTH was selected for analysis. Hematologic and biochemical variables were compared to their reference ranges at the time of sample collection (which varied over the study period) to define results as within, above, or below reference ranges. For urinalysis results, microscopic hematuria was defined as ≥10 red blood cells/hpf, and pyuria was defined as ≥5 white blood cells/hpf. Evidence of concurrent polyarthritis was noted; this evidence included either results of synovial fluid analysis documenting neutrophilic inflammation or at least 3 of the following physical examination findings: fever, stiff gait, lameness, joint swelling or effusion, or pain on joint palpation or manipulation. All renal histopathologic findings (obtained by renal biopsy or at necropsy) were recorded. Finally, last known outcome was determined based on medical records and recorded owner communications.

2.1 | Renal histopathology

Available biopsy specimens were classified as consistent or inconsistent with a diagnosis of immune complex glomerulonephritis (ICGN) using criteria similar to those previously published.[19] In brief, specimens submitted to the University of California, Davis Anatomic Pathology service, were fixed in 10% buffered formalin for histologic analysis. Paraffin-embedded tissues then were sectioned at 5 μm thickness and stained routinely with hematoxylin and eosin. Additional sections were prepared for examination by Masson's trichrome staining, periodic acid Schiff reaction, or Jones methenamine silver method. All microscopic sections were evaluated by a board-certified veterinary anatomic pathologist (F.C.M.). Where biopsy

specimens had been submitted to the International Veterinary Renal Pathology Service, available histopathologic images were reviewed retrospectively by the same pathologist (F.C.M.). Histologic features suggestive of a diagnosis of ICGN were hypercellularity in the peripheral aspects of glomerular lobules and the mesangium; glomerular basement membrane thickening, duplication, remodeling, spikes, and holes; intracapillary neutrophils; nuclear pyknosis; and mesangial matrix expansion. When available, evidence for the presence of glomerular immune complex deposits also was evaluated by transmission electron microscopy and immunofluorescence microscopy. Dogs with other findings were classified as non-ICGN cases.

2.2 | Statistical analysis

Descriptive statistics were calculated; normally distributed variables were expressed as mean ± standard deviation, and those not normally distributed were expressed as median (range). Categorical data were expressed as frequencies; values with a nonlinear distribution were analyzed categorically. Fisher's exact test was used to compare categorical variables. Conditional univariate logistic regression was used to estimate matched odds ratios and 95% confidence intervals (CI) relating individual blood variables to being diagnosed as a case versus a control. For histopathologic classification, the odds ratio was inestimable using conventional conditional logistic regression, and therefore exact conditional logistic regression was used. A specification link test for single-equation models (link test, Stata, version 11; StataCorp, College Station, Texas) was used to screen for specification errors when using linear quantitative variables. All analyses were performed using commercial statistical software (Stata, version 11; StataCorp).

3 | RESULTS

In all, 40 dogs with *Borrelia* C6-antigen seroreactive PLN were identified for inclusion, along with 78 temporally matched control dogs. Two of the cases had only 1 control dog identified because of insufficient numbers of control dogs evaluated between cases. Differences in categorical variables between cases and controls are shown in Table 1, and continuous variables are shown in Table 2.

Among seroreactive dogs, the most common breeds were Labrador Retrievers (13/40) or Labrador mixes (4/40), Golden Retrievers (4/40) or mixes (1/40), and Australian Shepherds (3/40) or mixes (1/40). Controls similarly were distributed across a variety of breeds, the most common of which were Labrador Retrievers (5/78) or mixes (1/78), American Cocker Spaniels (5/78) or mixes (2/78), Yorkshire Terriers (5/78), and Golden Retrievers (4/78). When Labrador and Golden Retrievers were considered together ("retrievers"; see Table 1), these breeds were overrepresented in the case group. This was true when purebreds were considered alone ($P = .001$) or when purebreds and mixes were considered together ($P < .001$).

The median duration of clinical signs before initial examination was 19.0 days for seronegative dogs and 18.5 days for seroreactive dogs ($P = .10$). Reported clinical signs were inappetence (29/78 controls, 31/40 cases, $P < .001$), lethargy or weakness (32/78 controls, 25/40 cases, $P = .049$), vomiting (20/78 controls, 27/40 cases, $P < .001$), weight loss (14/78 controls, 12/40 cases, $P = .18$), increased thirst and urination (12/78 controls, 2/40 cases, $P = .17$), diarrhea (10/78 controls, 3/40 cases, $P = .58$), joint pain or lameness (6/78 controls, 6/40 cases, $P = .37$), ascites or peripheral edema (8/78 controls, 0/40 cases, $P = .059$), and respiratory signs (4/78 controls, 2/40 cases, $P = 1.0$).

Blood pressure evaluation was performed in 68 of 78 seronegative and 39 of 40 seroreactive dogs. Results were considered consistent with the presence of systemic hypertension in 48 of 68 (70.6%) seronegative dogs and 33 of 39 (84.6%, $P = .20$) seroreactive dogs.

Low numbers of dogs overall had evidence of polyarthritis at the time of examination (8/40 cases, 7/78 controls, $P = .17$; Table 1). Physical examination findings consistent with polyarthritis were pain on joint palpation or manipulation (4/7 controls, 3/8 cases), joint swelling or effusion (2/7 controls, 4/8 cases), lameness (1/7 controls, 4/8 cases), stiff gait (2/7 controls, 3/8 cases), fever (1/7 controls, 2/8 cases), spinal pain (1/7 controls), and abnormal stance (1/7 controls). Polyarthritis was confirmed by identification of nonseptic, suppurative inflammation in synovial fluid specimens in 5 of 8 cases and 6 of 7 controls.

Complete blood count and urinalysis data were available for 39 of 40 cases and all controls, and serum biochemistry panel and UPCR results were available for all cases and controls (Tables 1 and 2). Mean hematocrit was lower (27.0% versus 38.2%, $P < .001$) and mean corpuscular volume (MCV) was higher (72.7 versus 70.1, $P = .005$) for cases as compared to controls, although mean MCV remained within reference ranges for both groups. White blood cell counts were nonlinearly distributed, but the proportion of dogs with leukocytosis or leukopenia did not differ between the 2 groups (Table 1). A few dogs in either group had increased numbers of band neutrophils (9/39 cases, 13/78 controls), which typically was mild. Lymphopenia and thrombocytopenia occurred more often in cases than in controls ($P = .006$ and $<.001$, respectively), and mean lymphocyte and platelet counts also were lower in case dogs ($P = .03$ and $<.001$, respectively). Where present, thrombocytopenia generally was mild to moderate (50 000-150 000 platelets/μL). However, 9 of 26 dogs had platelet counts ≤50 000/μL; 2 of these dogs were controls.

Nearly all dogs in the C6-seroreactive group (38/40) had a serum creatinine concentration above the reference range (>2.2 mg/dL), compared to just over half of the controls (41/78, $P = .002$; Table 1). Similarly, mean serum urea nitrogen concentration was higher in cases (101.8 mg/dL) as compared to controls (61.7 mg/dL, $P < .001$; Table 2). Serum phosphorus and potassium concentrations both were higher in cases as compared to controls ($P < .001$ and .04, respectively), whereas serum albumin concentration was lower in cases ($P = .004$).

Glucosuria, hematuria, and pyuria were more prevalent in cases than in controls ($P < .01$ for all; Table 1). Urine protein:creatinine ratios did not differ between the 2 groups. Results of aerobic bacterial urine culture were available for 38 of 40 cases and 74 of 78 controls and were positive in only 1 case dog with growth of $>10^5$ cfu/mL *Escherichia coli*.

Results of testing for other infectious diseases are shown in Table 3. One case dog seroreacted to *Ehrlichia canis* (1:100) and *Rickettsia rickettsii* (1:25). The other case dog that seroreacted to *R. rickettsii* had a titer of 1:320 with no other identified exposure to co-pathogens (negative for

TABLE 1 Results of analysis of categorical variables comparing 40 dogs with *Borrelia* C6-antigen seroreactive PLN and 78 dogs with *Borrelia* C6-antigen PLN. ICGN, immune-complex glomerulonephritis

	Borrelia C6 seroreactive	*Borrelia* C6 seronegative	OR	95% CI	*P* value
Age ≤8	27/40	44/78	1.69	0.74-3.90	.22
Male sex	15/40	42/78	0.50	0.22-1.12	.09
Intact	6/40	13/78	0.81	0.27-2.46	.72
Rectal temperature ≥102.5 (°F)	4/40	7/78	1.18	0.30-4.63	.81
Retriever					
Purebred	17/40	9/78	5.53	2.01-15.22	.001
Purebred or mix	22/40	10/78	7.22	2.70-19.30	<.001
Hypertension	33/39	48/68	2.05	0.75-6.59	.20
Polyarthritis	8/40	7/78	2.55	0.71-10.19	.17
ICGN	16/16	7/23	7.30	0.96-∞	.06
CBC					
Anemia	37/39	46/78	11.33	2.61-49.17	.001
Thrombocytopenia	26/38	9/75	14.55	4.36-48.54	<.001
Leukocytosis	20/39	27/78	2.00	0.90-4.23	.09
Leukopenia	3/39	3/78	2.00	0.40-9.91	.40
Neutrophilia	23/39	22/78	3.82	1.58-9.23	.003
Lymphopenia	18/39	17/78	3.79	1.46-9.87	.006
Biochemistry					
Creatinine ≥2.2	38/40	41/78	24.67	3.30-184.36	.002
Hyperphosphatemia	37/40	32/78	17.14	4.02-72.97	<.001
Hyperkalemia	11/40	12/78	2.50	0.88-7.11	.09
Hypoalbuminemia	38/40	63/78	4.56	0.98-21.35	.05
Hyperglobulinemia	6/40	8/77	1.55	0.51-4.69	.44
Urinalysis					
Glucosuria	19/39	10/78	9.33	2.71-32.14	<.001
Hematuria	19/39	16/78	4.32	1.68-11.11	.002
Pyuria	12/39	0/78	33.63	5.55-∞	<.001
Cylindruria	22/39	33/78	1.85	0.82-4.14	.14

Abbreviations: OR, odds ratio. CI, confidence interval.

heartworm antigen, and for antibodies to *Ehrlichia*, *Babesia*, and *Anaplasma*). Six case dogs seroreacted to *Anaplasma* antigens. Titers were determined for 4 dogs and were 1:640, 1:80, 1:20, and 1:20; none had other known exposures. The single control dog that seroreacted to *R. rickettsii* (1:160) also seroreacted to *Babesia vogeli* (titer 1:80). The 4 control dogs that seroreacted to *E. canis* had titers that ranged from 1:10240 to 1:327680; 2 of these dogs also seroreacted to *Anaplasma* spp.

The only comorbid disease identified in the case dogs was a hepatic mass of unknown type in 1 dog. Among control dogs, 6 dogs had neoplasia (2 with lymphoma, 2 with mast cell tumors, and 1 each with peripheral nerve sheath tumor, osteochondroma, osteosarcoma, and a complex splenic mass). Three control dogs had central nervous system disease (2 with myelopathies, and 1 with inflammatory brain disease), and 3 control dogs had endocrine disease (1 each with diabetes mellitus, pituitary-dependent hyperadrenocorticism, and inappropriate erythropoietin production). Other diseases in the control dogs included a chronic jejunal intussusception and a perivulvar abscess.

In the 14 days before examination at the VMTH, 31 of 40 case dogs and 48 of 78 control dogs were treated with ≥1 anti-infective agents (P = .10), and 11 of 40 cases and 24 of 78 controls were treated with an anti-inflammatory or immunosuppressive agent (P = .71; Table 4).

Renal biopsy, necropsy, or both was performed in 19 cases and 29 controls. Nine dogs (3 cases and 6 controls) were excluded from assessment of histopathology findings because of missing or non-diagnostic biopsy samples upon review. Renal specimens from all 16 case dogs were classified as consistent with ICGN, as compared to 7 of 23 specimens from controls (P = .06). The remaining control dogs had amyloidosis (6), glomerulosclerosis (4), or a variety of findings that were inconsistent with ICGN (6). If dogs with membranous glomerulonephritis (MGN) were removed from the ICGN category, 15 of 16 cases and 3 of 23 controls were classified as having ICGN (P = .06, OR, 7.58; 95% CI, 0.90-63.94).

The last documented outcome was recorded for all dogs. For cases, 13 of 40 dogs were documented as alive at a median of 72 days after

TABLE 2 Results of analysis of continuous variables comparing signalment, hematologic, serum biochemistry analysis, and urinalysis findings in 40 dogs with *Borrelia* C6-antigen seroreactive PLN and 78 dogs with *Borrelia* C6-antigen seronegative PLN

		Borrelia C6 seroreactive			*Borrelia* C6 seronegative					
Variable (unit)	**Ref. range**	**Range**	**Mean**	**Median**	**Range**	**Mean**	**Median**	**OR**	**95% CI**	***P* value**
Age (y)	NA	3.0-11.9	7.0	6.5	0.4-18.3	7.8	7.6	NL		
Body weight (kg)	NA	5.9-64.0	24.8	24.7	1.8-63.0	20.5	20.3	1.03	1.00-1.06	.09
Rectal temperature (°F)	99.0-102.5	98.0-104.5	101.0	101.0	98.3-104.1	101.2	101.2	0.88	0.64-1.22	.45
Duration of clinical signs (days)	NA	1–160	34.8	18.5	0-498	60.7	19.0	0.99	0.99-1.00	.10
CBC										
HCT (%)	40-55	10.9-47.1	27.1	28.5	14.0-62.1	38.3	37.1	0.89	0.84-0.95	<.001
MCV	65-75	62.5-85.8	72.7	72.7	59.2-80.2	70.1	70.4	1.2	1.05-1.33	.005
MCHC	33-36	31.2-36.4	34.0	33.9	27.8-37.8	34.4	34.6	0.80	0.59-1.09	.16
WBC (×1000)	6.0-13.0	4.4-44.1	16.0	13.4	4.4-58.8	13.2	10.2	NL		
Neutrophils (×1000)	3.0-10.5	3.5-40.5	13.7	11.1	2.7-55.7	10.3	7.6	NL		
Bands	Rare	0-2921	229.0	0	0-1353	97.6	0	1.00	1.00-1.00	.12
Lymphocytes (×100)	10-40	2.2-31.6	11.8	10.1	1.6-67.4	15.9	14.0	0.94	0.88-1.00	.03
Platelets (×10,000)	15-40	2.8-54.2	15.1	10.4	0.8-84.4	35.7	31.9	0.92	0.89-0.96	<.001
MPV	7-13	7.4-21.2	13.1	12.7	5.8-20.5	11.0	10.3	1.23	1.07-1.42	.005
Biochemistry										
Albumin	3.4-4.3	1.2-3.7	2.0	2.0	1.0-4.3	2.4	2.4	0.33	0.02-0.70	.004
Globulins	1.7-3.1	1.9-5.1	3.0	2.8	0.9-8.8	2.8	2.6	NL		
Sodium	143-151	129-162	147.4	146.5	135-168	147.6	147	0.99	0.91-1.07	.74
Potassium	3.6-4.8	3.2-7.0	4.8	4.8	3.2-6.5	4.5	4.6	1.73	1.02-2.93	.04
Calcium	9.6-11.2	7.9-14.1	9.8	9.8	7.7-12.0	10.0	9.9	0.90	0.65-1.25	.53
BUN (×10)	1.1-3.3	1.7-25.7	10.2	9.8	0.8-17.2	6.2	5.2	1.2	1.11-1.38	<.001
Creatinine	0.8-2.2	0.7-11.9	5.4	5.0	0.3-13.2	2.9	1.8	NL		
Phosphorus	2.6-5.2	4.3-26.0	14.5	13.5	0.9-18.6	6.7	5.6	1.33	1.17-1.51	<.001
Urinalysis										
Specific gravity	≥1.025	1.010-1.036	1.018	1.017	1.006-1.057	1.018	1.015	NL		
UPCR	≤0.4	3.8-27.1	13.8	12.9	5.0-64.4	14.6	11.4	0.99	0.95-1.03	.53

Abbreviations: BUN, blood urea nitrogen; CI, confidence interval; HCT, hematocrit; MCHC, mean cell hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; NL, nonlinear; OR, odds ratio; UPCR, urine protein:creatinine ratio; WBC, white blood cells.

TABLE 3 Results of testing for other infectious agents (using serology, PCR, or both) in dogs with PLN that were seroreactive (n = 40) or seronegative (n = 78) to *Borrelia* C6 antigen

	Borrelia C6 seroreactive (*# positive/ # tested*)	*Borrelia* C6 seronegative (*# positive/ # tested*)
Dirofilaria immitis	0/30	0/67
Ehrlichia canis	1/39	4/77
Anaplasma spp.	6/38	2/74
Rickettsia rickettsii	2/18	1/33
Leptospira spp. (titer >1:800)	0/24	3/26
Babesia spp.	0/8	2/21
Bartonella spp.	0/1	0/5
Coccidioides immitis	…	1/4

TABLE 4 Medications administered to dogs with PLN in the 14 days before initial examination

	Borrelia C6 seroreactive (n = 40)	*Borrelia* C6 seronegative (n = 78)
Antibiotics		
Penicillins/cephalosporins	17	30
Fluoroquinolones	11	17
Nitroimidazoles	1	11
Tetracyclines	18	9
Macrolides/lincosamides	0	3
Trimethoprim/sulfa	0	1
Antifungals	0	2
Anti-inflammatories		
Glucocorticoids[a]	5	10
NSAIDs[a]	5	15
Other immunosuppressive drugs	2	2

Abbreviation: NSAIDs, nonsteroidal anti-inflammatory drugs.
[a]Includes oral and injectable forms; topical ophthalmic formulations excluded.

diagnosis (range, 2-706 days). Of these 13 dogs, 2 had progression of their renal disease, 5 had improved or stable disease, and 6 of 13 had unknown renal status. The remaining 27 dogs died or were euthanized at a median of 7 days after diagnosis (range, 0-965 days); 25 of 27 of these dogs had progressive renal disease. The other 2 dogs had stable renal disease and died of unrelated causes (lymphoma, hepatic mass). Among controls, 40 dogs were alive a median of 64 days after diagnosis (range, 1-1619 days); 9 were known to have progressive renal disease, 10 had improved or stable disease, and 21 had unknown renal status. The remaining 38 dogs died or were euthanized at a median of 42 days after diagnosis (range, 1-2341 days); 36 of 38 had progressive renal disease. One of the other 2 dogs was euthanized for upper airway obstruction, and the other was reported to have died with no further information provided. There was no difference among groups in days to last known outcome (P = .12) or in overall number of dogs experiencing death or euthanasia (P = .08).

4 | DISCUSSION

Our results suggest that a unique set of clinicopathologic abnormalities is present in dogs with Lyme C6 seroreactive PLN as compared to other types of PLN. When Lyme nephritis was first described in dogs, the existence of an association between seroreactivity and nephritis was questioned because of the high prevalence of subclinical seroreactivity to *B. burgdorferi* in endemic areas. Although assays that target the Lyme C6 antigen have overcome problems associated with vaccine and nonspecific cross-reactivity that were observed with *Borrelia* immunofluorescent antibody assays, widespread subclinical infection together with the prolonged (2-5 month) incubation period means that diagnosis of Lyme polyarthritis in dogs still relies upon a combination of positive serology, exclusion of other causes of polyarthritis, and rapid response to treatment with doxycycline. Exclusion of other causes is difficult in dogs with polyarthritis because the primary noninfectious differential diagnosis is idiopathic immune-mediated disease. Diagnosis of Lyme nephritis presents additional challenges, because the time course and underlying cause of PLN typically are difficult to determine even with renal histopathologic examination, and Lyme nephritis usually does not respond to treatment with doxycycline. Currently, diagnosis most often is based on the presence of PLN, exclusion of other causes, and a positive C6 antibody titer, with or without evidence of membranoproliferative glomerulonephritis on histopathology of renal biopsy specimens; no study has consistently detected spirochete DNA or antigen within renal biopsy specimens.[20,21] In highly endemic areas, the high prevalence of seroreactivity (>80%) means that a subset of C6 antibody-positive dogs likely have PLN due to other causes. Therefore, identification of specific clinicopathological abnormalities that might increase suspicion for Lyme nephritis would be valuable for diagnosis. Because the overall prevalence of C6-seropositivity to *B. burgdorferi* in our region is low (1.2%),[2] our patient population was well suited to a case-control study because of the ready availability of a number of temporally matched control dogs.

Although we could not prove that all dogs with antibodies to the C6 antigen had Lyme nephritis, our findings provide strong support that in northern California, dogs with PLN that also have evidence of exposure to *Borrelia* have a distinct clinical syndrome that separates them from dogs with PLN that have no evidence of exposure to *Borrelia*. Variables that were more likely to be present in dogs suspected to have Lyme nephritis were thrombocytopenia, azotemia, hyperkalemia, hyperphosphatemia, glucosuria, hematuria, and pyuria. In our study, 20% of dogs that were C6-seroreactive had physical examination or arthrocentesis findings that either suggested or confirmed the presence of polyarthritis, which is similar to the previous report of concurrent lameness or polyarthritis in 26% of dogs with Lyme nephritis.[18] However, most dogs with C6-positive PLN

did not have signs of polyarthritis at the time of evaluation. The prevalence of fever was low and not associated with seroreactivity.

Nearly 70% of seroreactive dogs in our study were thrombocytopenic, as previously described.[18,22] Thrombocytopenia has been reported in association with Lyme disease in humans, and an autoimmune mechanism has been proposed based on the detection of antiplatelet autoantibodies.[23,24] This finding has been considered controversial because of the possibility of coinfection with *Anaplasma phagocytophilum* (although more recent studies have not shown this finding to be a meaningful confounder[25]), and, in general, thrombocytopenia is considered uncommon to rare in humans with acute Lyme disease.[26] Detectable antibody titers to *Anaplasma*, *Ehrlichia*, or *Rickettsia* antigens (alone or in combination) were present in 7 of 40 case dogs, and the possibility of co-pathogens causing some of the observed clinicopathologic changes cannot be ruled out (although of these 3 agents, only *Ehrlichia* causes chronic, persistent infections that might be associated with PLN, and only 1 case dog seroreacted to *Ehrlichia* with an antibody titer of 1:100). Testing for vector-borne pathogens was not comprehensive in most of the cases, and convalescent serology was performed inconsistently. Therefore, undetected coinfections could not be excluded.

The higher prevalence of azotemia, hyperkalemia, and hyperphosphatemia in the cases compared with the controls is consistent with the previous study that described *Borrelia*-seroreactive PLN as severe and rapidly progressive.[18] This might also explain why higher percentages of case dogs had signs of vomiting, lethargy, weakness, or inappetence reported at the time of initial examination. The presence of azotemia alone cannot be taken to imply direct renal damage given that prerenal azotemia as a consequence of dehydration cannot be excluded. The higher prevalence of glucosuria, hematuria, and pyuria in the cases, however, suggests that more marked tubuloglomerular damage may have been present in cases than in controls. Unexpectedly, pyuria was detected only in the C6-seroreactive dogs, and not in the control dogs, and therefore the presence of pyuria in dogs with PLN may be helpful to support a diagnosis of Lyme nephritis in highly endemic areas. Anemia, neutrophilia, and lymphopenia also were more common in affected dogs in our study.

The largest case study of *Borrelia*-seropositive PLN to date was from the northeastern United States.[18] In that study, only 18 of 49 dogs were tested for antibodies to *Borrelia* (using indirect immunofluorescence, ELISA, or Western blot). Glomerular lesions were suspected to be associated with Lyme disease on the basis of positive serology results in all tested dogs, residence in a Lyme-endemic area, and a previous case report that described similar histopathologic findings.[27] More than half of the dogs in that study were ≤5 years of age. No association with age was seen in our study when the control group was used for comparison, and overall, a lower percentage of dogs in our study (29/118, 25%) were ≤5 years of age. Labrador and Golden Retrievers comprised over 40% of affected dogs in both our study and the previous study (24/49 cases), where the ORs for Labrador and Golden Retrievers as compared to the hospital background population were 6.4 and 4.9, respectively.[18] Retrievers also comprised a large proportion of dogs suspected to have Lyme nephritis in a subsequent study (21/58 [36%] dogs), but no comparison to a reference population was made.[21] The reason for the breed predisposition is unclear; possible explanations include increased recreation in tick-infested areas by these breeds or an actual genetic predisposition. Large-scale screening of Labrador and Golden Retrievers has not yielded any evidence of an association between microalbuminuria and Lyme serology status.[28] In humans, certain histocompatibility leukocyte antigen (HLA) types, including HLA-DR4 and HLA-DR2, are more frequent among patients who develop chronic Lyme arthritis.[29-32] Although a wide range of proteins enter the HLA-DR pathway of antigen presentation, the specific HLA-DR genotype that is present in an individual appears to be largely responsible for its peptide repertoire.[33] There is increasing evidence that presentation of novel autoantigens, particularly derivatives of endothelial cell growth factor, matrix metalloproteinase-10, and apolipoprotein B-100, by HLA-DR molecules to CD4+ T cells is responsible for the chronic inflammation seen in non-antibiotic-responsive Lyme arthritis in susceptible individuals.[33-37] It is possible that a similarly abnormal immune response could be responsible for ongoing inflammation in retriever breeds, leading to secondary immune-mediated glomerulonephritis. Further investigation is needed to clarify the role of genetics and specific immune responses in dogs with borreliosis.

Where available, renal histopathology was consistent with ICGN in all case dogs in our study. We chose to include MGN in this category, even though Lyme nephritis classically is described as membranoproliferative glomerulonephritis. Removal of the MGN classification only eliminated 1 case dog from analysis and did not meaningfully alter the statistical analyses. Interestingly, dogs with MGN previously have been described in association with putative Lyme nephritis,[18] but not all dogs of that report were tested for Lyme seroreactivity.

Our study had several limitations. The diagnosis of polyarthritis was based on clinician descriptions, with or without corroborating synovial fluid analysis, and subclinical polyarthritis may have gone undetected in some cases. Treatments administered before the time of examination at our hospital varied widely. Although similar percentages of case and control dogs were treated either with anti-infective or anti-inflammatory medications before examination, their effects on clinicopathologic variables could not be completely eliminated. It is possible that clinical abnormalities detected could have been attributable to etiologies other than *Borrelia* infection, because the lack of consistently comprehensive testing in all dogs precluded exclusion of other causes of disease. Nevertheless, diagnostic evaluations generally were thorough given that our institution is a tertiary referral hospital. Confirmation of active infection in seropositive dogs was not possible, and some of these dogs may have been seropositive as a result of previous subclinical exposure or infection with subsequent development of PLN from unrelated causes. However, the overall prevalence of seroreactivity in the geographic region in which the majority of the dogs in our study resided is low,[2] and our experience is that, when tested, dogs seen at our hospital with clinical abnormalities that are not consistent with those described for Lyme disease rarely seroreact to *Borrelia* C6 antigen. The C6 assay is sensitive (84.2%-96.7%) and specific (96.1%-98.8%) as compared to Western blotting.[38-40] Although false-positive test results are possible in low-prevalence populations, the selection of a population of at-risk dogs (ie, those with evidence of PLN) likely increased the positive

predictive value of *Borrelia* testing. Lastly, for the diagnosis of PLN, an arbitrary value of UPCR >5 was selected; occasionally tubular disease alone may cause UPCRs of this magnitude, and biopsy confirmation of glomerulonephritis was not obtained in some cases. However, this cut-off value is similar to criteria that have been used in other studies[19] and was intentionally selected to be substantially higher than American College of Veterinary Internal Medicine guidelines (describing glomerular proteinuria as persistent UPCR ≥2.0[41]) in order to decrease the chance of including dogs with tubular proteinuria. A single seropositive dog with a positive urine culture, which may have confounded UPCR results, also was included, but UPCR for that dog remained increased (>15) after treatment of the infection and subsequent negative urine culture results.

In conclusion, we have provided additional evidence that Lyme nephritis has clinical features that distinguish it from other causes of PLN. Factors that may add weight to a diagnosis of Lyme nephritis in seroreactive dogs with PLN include disease in a retriever or retriever mix; biochemical evidence of azotemia, hyperkalemia, and hyperphosphatemia; findings of thrombocytopenia, anemia, or neutrophilia on CBC, and the presence of hematuria, glucosuria, and pyuria despite negative urine culture. Concurrent polyarthritis was not found to be a distinguishing characteristic in our study, although subclinical polyarthritis may have been present in some dogs from each group but not recognized. Until further evidence is available to guide appropriate treatment, given the recognized poor prognosis associated with this syndrome, combinations of these identified features in C6-seropositive dogs should prompt aggressive treatment with both doxycycline and immunosuppressive drug treatment.[22,42]

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CONFLICT OF INTEREST DECLARATION

Jane Sykes serves as Associate Editor for the *Journal of Veterinary Internal Medicine*. She was not involved in review of this manuscript.

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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