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Bartonella spp. seroepidemiology and associations with clinicopathologic findings in dogs in the United States

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Abstract

Background: Improved understanding of *Bartonella* spp. serology in dogs may aid clinical decision making.

Objective: Describe demographic and geographic patterns of *Bartonella* spp. seroreactivity in dogs, and describe hematologic and serum biochemical abnormalities in *Bartonella* spp. seroreactive and nonseroreactive dogs.

Animals: Serum samples from 5957 dogs in the United States, previously submitted to IDEXX Reference Laboratories.

Methods: Serum was tested using 3 indirect ELISAs for *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, and *B. koehlerae*. Complete blood count and serum biochemistry panel results were reviewed retrospectively.

Results: Overall, 6.1% of dogs were *Bartonella* spp. seroreactive. Toy breeds were less likely to be seroreactive (3.9%) than mixed breeds (7.5%; adjusted odds ratio [aOR], 0.48; 95% confidence interval [CI], 0.32-0.72), and dogs <1 year old were less likely to be seroreactive (3.4%) than dogs 1 to 5.5 years of age (7.3%; aOR, 0.42; 95% CI, 0.23-0.72). Dogs in the West South Central (9.8%) and South Atlantic (8.8%) regions were more likely than dogs elsewhere in the United States to be seroreactive (aOR, 2.22; 95% CI, 1.31-3.87; aOR, 2.44; 95% CI, 1.38-4.36).

Conclusions and Clinical Importance: Demographic and geographic findings for *Bartonella* spp. exposure were broadly comparable to previously reported patterns.

KEYWORDS
bartonellosis, canine, seroreactivity, vector-borne, zoonoses

1 | INTRODUCTION

Members of the genus *Bartonella*, gram-negative hemotropic and endotheliotropic bacteria, are important emerging pathogens worldwide.[1-3] Domestic dogs can be infected with at least 10 *Bartonella* species.[3,4] *Bartonella vinsonii* subsp. *berkhoffii* (*Bvb*), *Bartonella henselae*

Abbreviations: aOR, adjusted odds ratio; *Bh*, *Bartonella henselae*; *Bk*, *Bartonella koehlerae*; *Bvb*, *Bartonella vinsonii* subsp. *berkhoffii*; Ca×P, calcium-phosphorus product; CI, confidence interval; CVBD, canine vector borne diseases; IFA, indirect fluorescent antibody assay; OR, odds ratio; PCR, polymerase chain reaction.

(*Bh*), and *Bartonella koehlerae* (*Bk*) represent the most common species that infect dogs in North America.[5] All 3 of these species are considered pathogenic in cases of endocarditis in dogs,[6-9] and have been associated with a wide variety of other clinical abnormalities such as vasoproliferative diseases, vasculitis, myocarditis, polyarthritis, granulomatous disease (lymphadenitis, rhinitis, hepatitis), epistaxis, and neurological manifestations.[10-19]

Bartonella species are primarily arthropod transmitted,[20-22] but to date no definitive vector has been identified for natural transmission of *Bartonella* to or among dogs. On the basis of case reports,[15,21,23-25] serosurveys,[26-34] surveys of arthropod vectors,[35-39] and experimental data,[40-42] ticks and fleas have been proposed as potential vectors for *Bartonella* spp. transmission in dogs. Although *Bartonella* spp. seroreactivity is not diagnostic for *Bartonella* spp. infection, evaluation of seroreactivity can be used as a marker of *Bartonella* spp. exposure in dogs. *Bartonella* seroepidemiological studies therefore can provide important information about temporo-spatial distribution of pathogen exposure, and regional differences in *Bartonella* spp. seroreactivity indirectly can implicate potential arthropod vectors.

Previous epidemiologic studies of *Bartonella* spp. seroreactivity in dogs have examined demographic and geographic patterns, as well as coexposure with other vector-borne diseases of dogs (CVBDs), but have not comprehensively examined associations with clinicopathologic abnormalities.[26-34] Additionally, previous seroepidemiologic studies have used the indirect fluorescent antibody test (IFA), which is the current gold-standard test, to determine seroreactivity status. Because IFA is labor-intensive, expensive, can only be performed at specialty laboratories, and has low sensitivity when compared to *Bartonella* spp. When compared to amplicfication of *Bartonella* spp. DNA using PCR from blood or tissue, a need exists for alternative serologic tests to evaluate for potential *Bartonella* spp. exposure in dogs.[43-46]

Improved understanding of *Bartonella* spp. seroreactivity patterns in dogs may therefore aid clinical decision making, as well as enhance current understanding of naturally-occurring transmission dynamics. We aimed to determine demographic and geographic factors associated with *Bartonella* spp. seroreactivity detected using a novel bacterial whole-cell ELISA in a large population of dogs across the United States. A secondary aim was to describe the types and frequencies of clinicopathologic abnormalities in *Bartonella* seroreactive dogs. The null hypothesis was that there would be no significant demographic or geographic associations with *Bartonella* spp. seroreactivity in dogs.

2 | MATERIALS AND METHODS

2.1 | Study design, setting, and participants

This retrospective study used serum samples collected from dogs and submitted to IDEXX Reference Laboratories for routine diagnostic testing by veterinarians throughout the United States during the period from May 1, 2016 through August 31, 2016. Serum samples were selected sequentially such that all samples with an adequate volume remaining after requested diagnostic testing was performed were included, with a goal to test 8000 samples. At the end of the sample

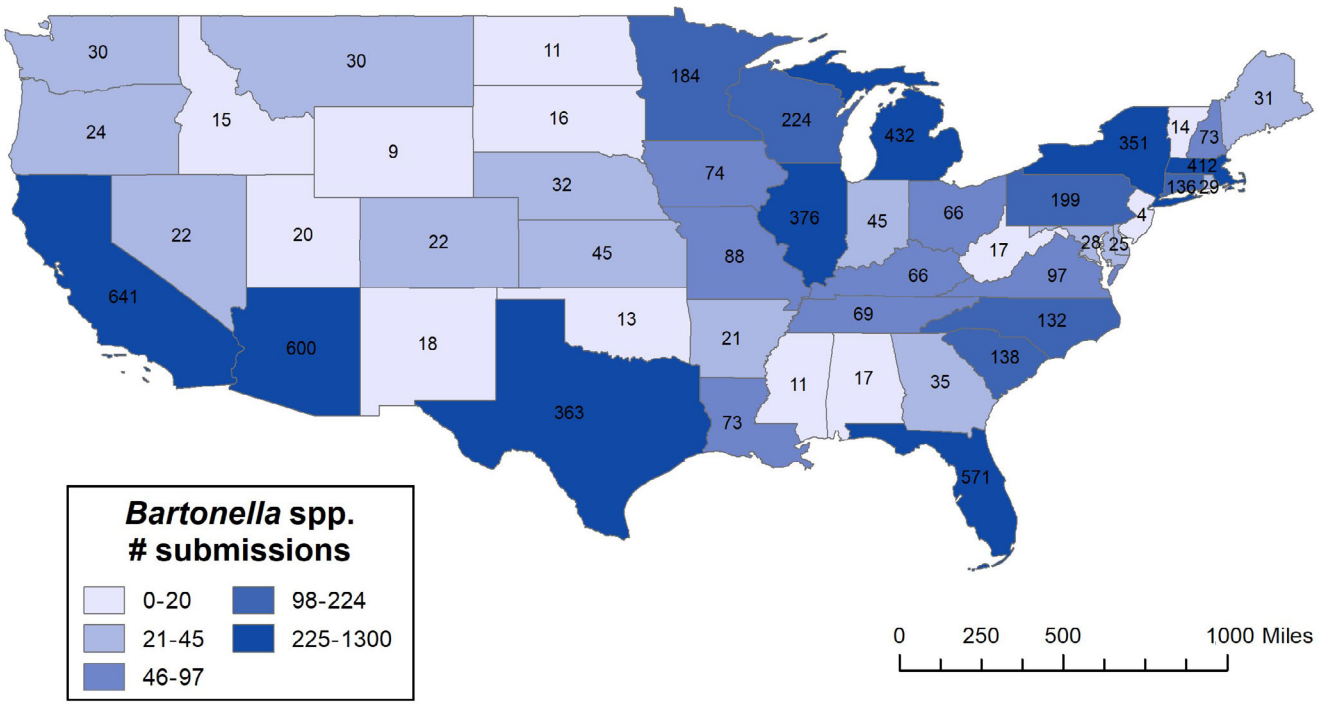

FIGURE 1 Map showing the state-level location of each included dog. Locations were based on the submitting veterinary hospital, and aggregated to the state level. Numbers inside each state indicate the total number of samples from that state

collection period, 5957 samples with adequate volume were available. If multiple samples from a single dog were available, only the first sample was used. All serum samples were stored at 2 to 8°C for a 7-day holding period, after which they were shipped frozen to investigators. Throughout the study, the samples were stored frozen (−20°C) and subjected to a maximum of 4 freeze-thaw cycles.[47,48] All samples were acquired with approval from the IDEXX Animal Welfare Committee (documentation available upon request).

TABLE 1 Demographic characteristics of study population, and proportion of dogs seroreactive to any *Bartonella* spp. for each demographic and geographic variable

	Total tested	# seroreactive (%)	*P*-value
Age	5957		
Puppy*	132	3 (2.3)	.03*
Junior*	265	8 (3)	.01*
Adult (Ref)	1254	91 (7.3)	Ref
Mature	1909	112 (5.9)	.15
Senior/geriatric	2365	144 (6.1)	.21
Not reported	32	4 (12.5)	.5
Sex	5957		
Female (Ref)	3024	188 (6.2)	Ref
Male	2899	172 (5.9)	.7
Not reported	34	2 (5.9)	.56
Breed group	5957		
Mix (Ref)	1161	87 (7.5)	Ref
All pure breeds	4494	252 (5.6)	–
Herding	520	35 (6.7)	.68
Sporting	1258	77 (6.1)	.21
Working	442	26 (5.9)	.25
Terrier	653	38 (5.8)	.12
Hound	411	23 (5.6)	.16
Nonsporting	399	21 (5.3)	.11
Toy*	811	32 (3.9)	<.001*
Not reported	302	23 (7.6)	.95
Region	5957		
West South Central*	470	46 (9.8)	.003*
South Atlantic*	1043	92 (8.8)	.004*
New England	695	44 (6.3)	.15
Middle Atlantic	554	31 (5.6)	.37
Mountain	736	40 (5.4)	.26
East North Central	1143	54 (4.7)	.82
East South Central	163	7 (4.3)	.86
West North Central	450	19 (4.2)	.94
Pacific (Ref)	703	29 (4.1)	Ref
Month received	5957		
May (Ref)	1278	80 (6.3)	Ref
June	2056	143 (7)	.87
July	1694	95 (5.6)	.99
August	929	44 (4.7)	.89

Note: *P*-values for each level within each category based on multivariable logistic regression.

*Statistically significant difference among proportions seroreactive in each category (baseline category indicated by "Ref"). Statistical significance considered when $P < .05$.

2.2 | Variables and data sources

The ELISA seroreactivity against any 1 or more *Bartonella* spp. was the main outcome of interest; secondary analyses also were performed for seroreactivity to *Bh* or *Bvb* specifically. A sample was considered *Bartonella* spp. ELISA seroreactive if it was seroreactive ≥1 of the 3 species ELISAs. The following items are presented in Supporting Information S1: (a) a description of the IFA-characterized samples used to develop the ELISAs; (b) a description of the culture conditions used to propagate organisms and the purification of whole cell lysates from those cultures; (c) ELISA plate coating and optimization; (d) assay protocol; (e) criteria used to determine whether samples were seroreactive; and (f) characterization of ELISA performance in terms of sensitivity and specificity compared to IFA.

Data for potential confounders and explanatory variables were retrieved retrospectively from IDEXX Reference Laboratory records for each dog with a serum sample enrolled for *Bartonella* ELISA testing. Individual dog data was deidentified. This information was reported by veterinarians on routine sample submission forms. Demographic information collected for each dog included: sex (male vs female; neuter status was not provided), age, and breed group (mixed vs purebred, with pure breeds further categorized by American Kennel Club [AKC] breed group), geographic region of origin, and month of submission. Ages were categorized as puppy (≤6 months), junior (7-12 months), adult (13-65 months), mature (66-113 months), and senior (≥114 months). Breed group was determined from the reported breed, and first was categorized into mixed breed or purebred, and then the purebred dogs were further divided by AKC breed group category. Mixed breed dogs with a predominant breed reported were categorized in the mixed breed group (eg, Labrador retriever mix categorized as mixed breed group). Dogs for which provided demographic information was incomplete (missing sex, age, or breed) were not excluded; missing demographic information was categorized as "not reported." The veterinary hospital or clinic address provided to IDEXX was used to categorize each sample with 2 different geographic variables: state and US Census region. When available, CBC, serum biochemistry, as well as any other concurrently submitted laboratory tests, also were collected from IDEXX Reference Laboratories. For each of these tests, results were categorized as low, normal, or high based on the reference intervals previously established by IDEXX (Table S2).

2.3 | Bias and study size

Because the dogs included in the study represent a convenience sample of blood submitted for diagnostic purposes, the distribution was

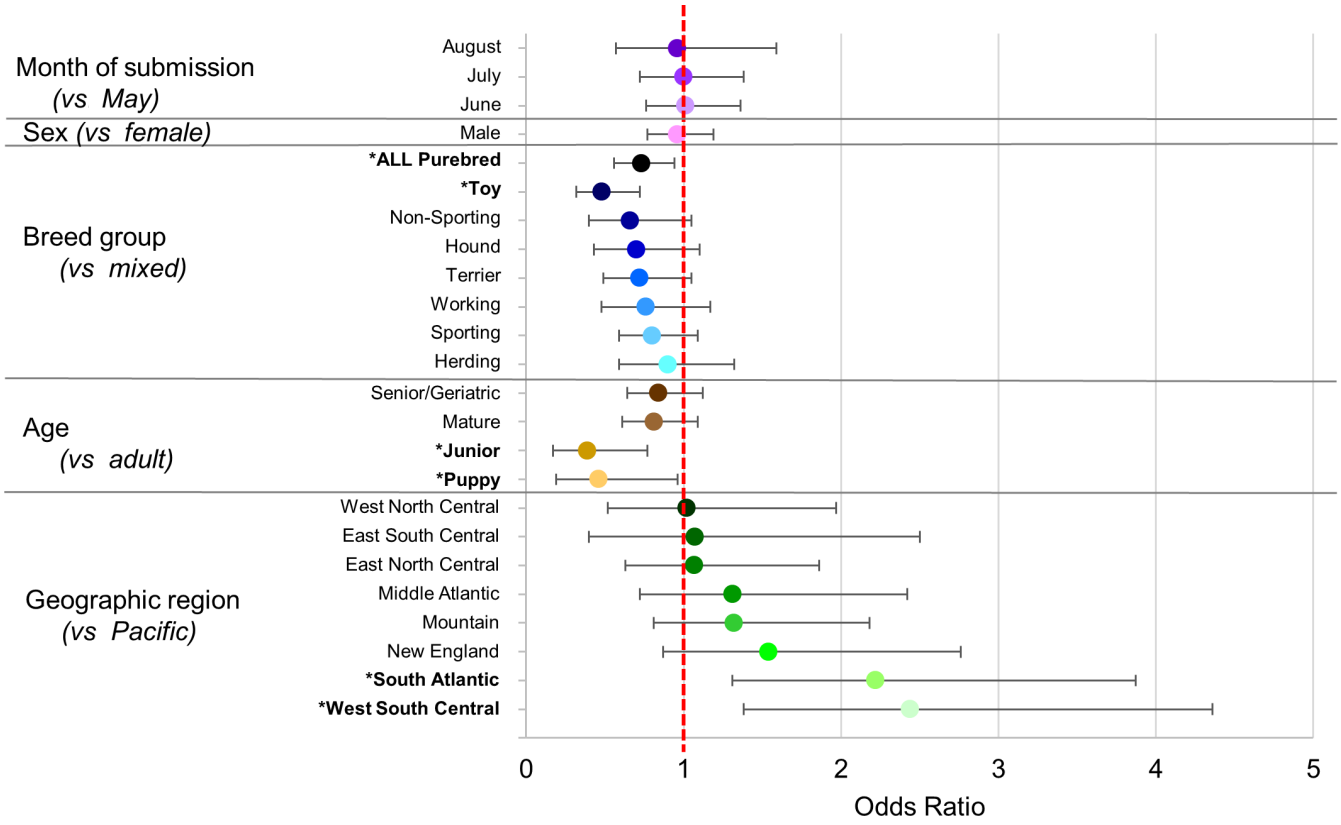

FIGURE 2 Associations between demographic/geographic variables and *Bartonella* spp. seroreactivity. Points represent aORs (colors show variable type: purple = month, pink = sex, blue = breed group, orange = age, green = region) with error bars showing 95% CIs. Statistical significance considered when $P < .05$, shown with * in bold text. Red vertical broken line indicates an aOR = 1

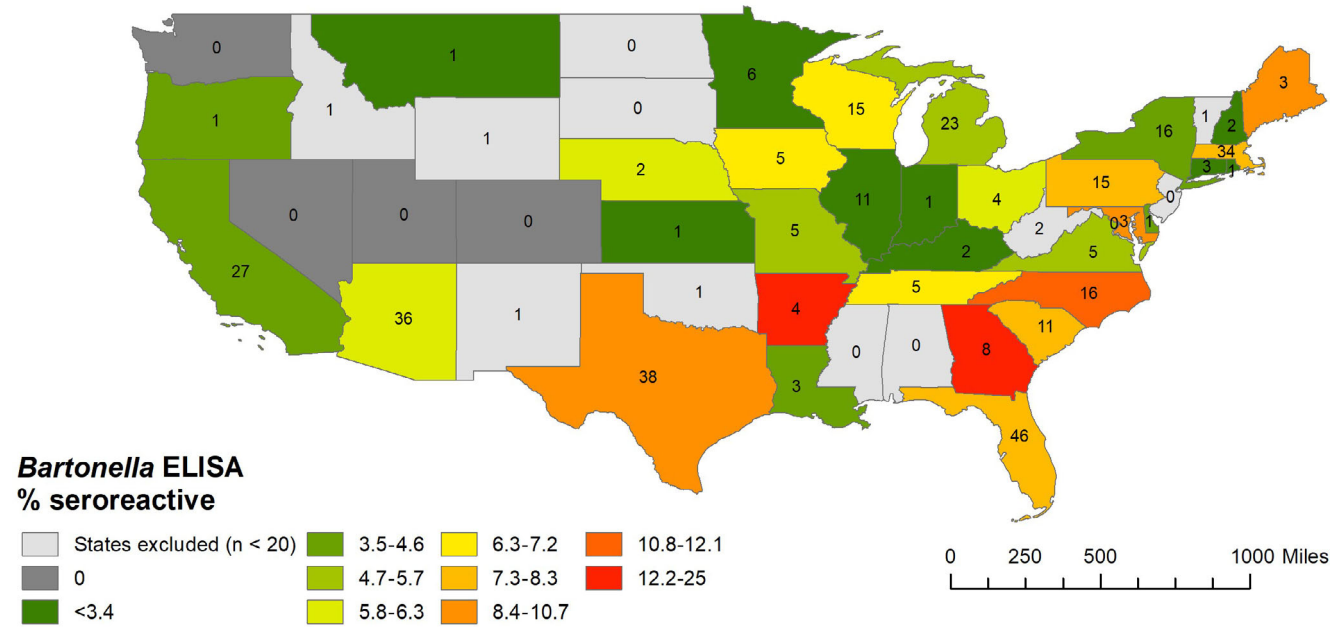

FIGURE 3 Map of *Bartonella* spp. ELISA seroreactivity in dogs, by state. Numbers in each state indicate the number of ELISA seroreactive serum samples from that state; colors indicate the percentage of serum samples that were seroreactive. Percentages for states with fewer than 20 serum samples tested are not shown (indicated with light gray)

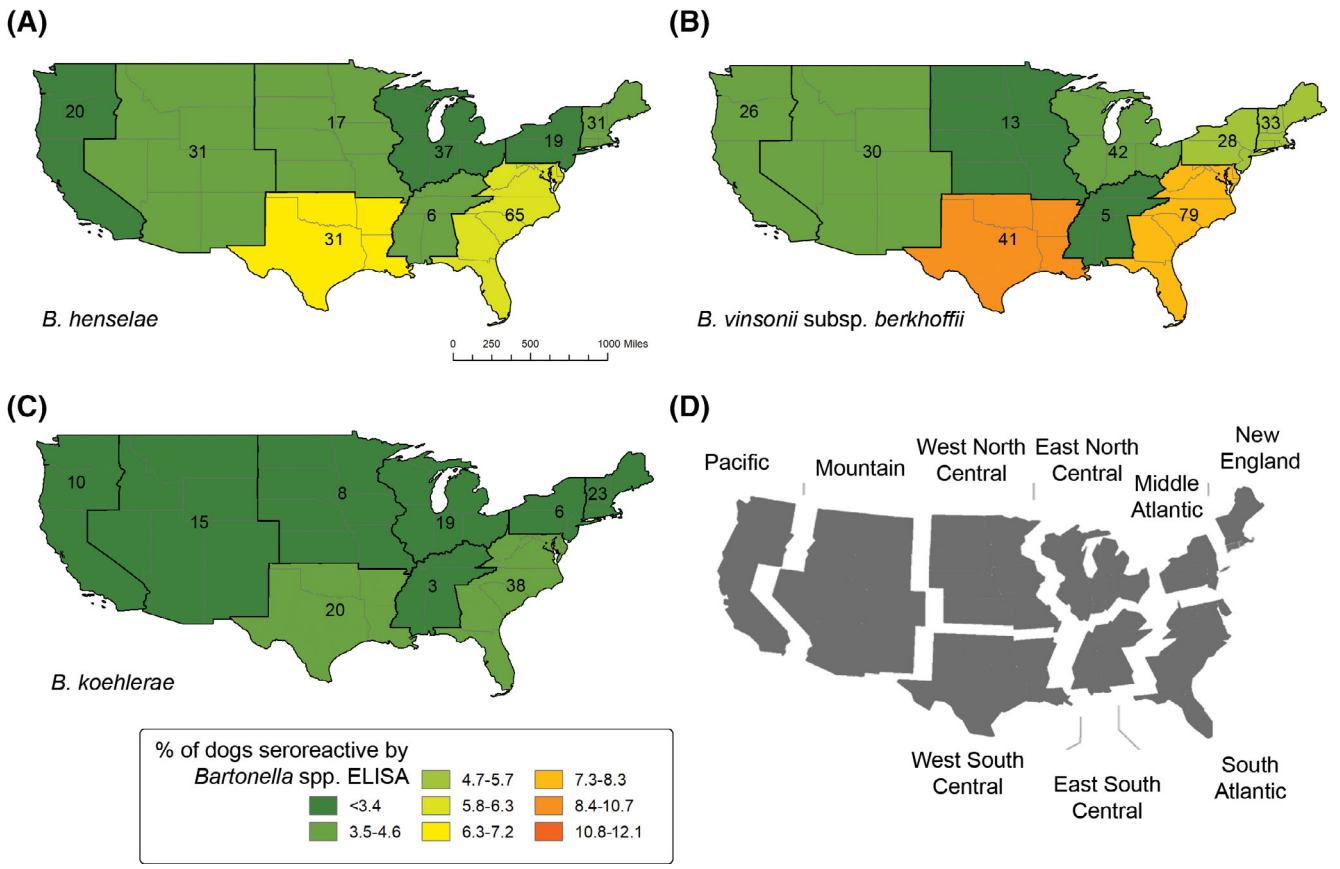

FIGURE 4 Maps of *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, and *B. koeherlae* ELISA seroreactivity in dogs, by census region. Numbers in each census region indicate the total number of seroreactive samples in that region; colors indicate the percentage of serum samples from that region that were seroreactive for each *Bartonella* species. (A) *B. henselae*, (B) *B. vinsonii* subsp. *berkhoffii*, (C) *B. koehlerae*. (D) States included in each census region

not uniform across geographic regions of the United States (Figure 1). In addition, because samples all were submitted for diagnostic testing, a bias may exist toward sicker dogs that are more likely to have diagnostic testing performed. Data for the explanatory variables was collected retrospectively, so there were incomplete results for demographic information, CBC, and serum biochemistry for a small percentage of dogs included in the study (Table 1). The reason for blood testing, medical and travel history, as well as any illnesses (infectious or otherwise) or exposure to CVBDs, for the dogs making up the sample is unknown, because this information is not routinely provided to the diagnostic laboratory when requesting these tests.

Possible bias due to demographic variables (age, sex, breed group), geographic location, or temporal factors (month of submission) were addressed by inclusion of these variables in the logistic regression model for each explanatory variable of interest.

2.4 | Statistical methods

All analysis was done using R v. 3.6.1 (www.R-project.org).[49] Descriptive statistics were obtained for each demographic, geographic, and clinicopathologic variable. Differences between seroreactive and nonseroreactive dogs were calculated using chi-squared tests.

To assess the association between each demographic and geographic variable and *Bartonella* spp. seroreactivity, we used a multivariable logistic regression model including age, sex, breed group, region, and month of sample submission. Adjusted odds ratios (aORs) and 95% confidence intervals (95% CIs) were estimated for each demographic and geographic variable from this model. The same procedures were used to assess associations with seroreactivity to *Bh*, *Bvb*, and *Bk* individually (using seroreactivity to *Bh*, *Bvb*, or *Bk* as the dependent variable). Statistical significance was set at $P < .05$ unless otherwise indicated.

Maps were created using ArcGIS (ArcMap v. 10.6.1, Environmental Systems Research Institute, Redlands, California). Boundaries were created from publicly available data from the US Census Bureau,[50] using the North American Datum (NAD) 1983 geographic coordinate system with Geodetic Reference System (GRS) 1980 spheroid.

3 | RESULTS

Serum samples from 5957 dogs were included in the study. Demographic characteristics of the included dogs are shown in Table 1,

TABLE 2 Proportion of dogs seroreactive to any *Bartonella* spp. for CBC variables

CBC variable	Categorical result	Total	# seroreactive (%)
HCT		4166	
	Normal	3562	206 (5.8)
	High	169	7 (4.1)
	Low	435	38 (8.7)
MCHC		4129	
	Normal	3576	200 (5.6)
	High	132	8 (6.1)
	Low	421	40 (9.5)
NP		4132	
	Normal	3709	213 (5.7)
	High	349	25 (7.2)
	Low	74	10 (13.5)
Eos		4133	
	Normal	3695	208 (5.6)
	High	159	13 (8.2)
	Low	279	27 (9.7)
Reticulocyte			
	Normal	2947	179 (6.1)
	Nonregenerative anemia	301	23 (7.6)
	Regenerative anemia	126	13 (10.3)
	Reticulocytosis	758	34 (4.5)

Note: The total number of dogs tested for each CBC variable is shown in the "Total" column, and differences reflect missing data due to technical limitations of the CBC.

TABLE 3 Proportion of dogs seroreactive to any *Bartonella* spp. for serum chemistry variables

Chem variable	Categorical result	Total	# seroreactive (%)
Alb		4148	
	Normal	3533	198 (5.6)
	High	64	3 (4.7)
	Low	551	53 (9.6)
Globs		4132	
	Normal	3663	194 (5.3)
	High	386	52 (13.5)
	Low	83	7 (8.4)
ALP		4319	
	Normal/Low	3232	210 (6.5)
	High	1087	48 (4.4)
BUN		4331	
	Normal	3879	222 (5.7)
	High	344	29 (8.4)
	Low	108	8 (7.4)
Creat		4263	
	Normal	3952	222 (5.6)
	High	225	23 (10.2)
	Low	86	8 (9.3)
SDMA		4309	
	Normal	3793	210 (5.5)
	High	516	45 (8.7)
Ca×P		3174	
	Normal	3057	180 (5.9)
	High	117	14 (12)

Note: ALP has combined normal and low category because there were only 4 dogs with ALP below the reference range. The total number of dogs tested for each serum chemistry variable is shown in the "Total" column, and differences reflect missing data due to technical limitations of the testing.

and a map of the geographic location for each included dog is shown in Figure 1. The overall proportion of dogs seroreactive to any of the 3 *Bartonella* spp. was 6.1% (362/5957). The most common species to which dogs were seroreactive was *Bvb* (5.0%), followed by *Bh* (4.3%) and *Bk* (2.4%). Of the 362 *Bartonella* spp. seroreactive dogs, 131 (36%) were ELISA seroreactive to all 3 *Bartonella* species.

When considering demographic factors, age, breed group, and region were independently associated with *Bartonella* ELISA seroreactivity (see Table S1 for model results). Percentages of dogs seroreactive for each demographic and geographic variable are shown in Table 1, and aORs and 95% CIs for each demographic and geographic variable are shown in Figure 2. Dogs <1 year of age were less likely to be seroreactive (2.8%) than dogs 1 to 5.5 years of age (6.0%; aOR, 0.35; 95% CI, 0.18-0.64); when specific age categories were considered, junior (7-12 months) and puppy (≤ 6 months) dogs had significantly lower seroreactivity against any *Bartonella* spp. compared to adult dogs (aOR, 0.39; 95% CI, 0.17-0.76 and aOR, 0.28; 95% CI, 0.07-0.77, respectively). Purebred dogs had significantly lower seroreactivity against any *Bartonella* spp. compared to mixed breeds (aOR, 0.73; 95% CI, 0.56-0.94), and when specific AKC breed groups were considered, toy breeds had significantly lower seroreactivity against any *Bartonella* spp. compared to mixed breeds (aOR, 0.49; 95% CI, 0.32-0.74). When considering geographic region, dogs from the South Atlantic (aOR, 2.22; 95% CI, 1.30-3.86) and West South Central (aOR, 2.38; 95% CI, 1.35-4.27) census regions had significantly higher seroreactivity against any *Bartonella* spp. compared to the Pacific census region. A map of *Bartonella* spp. seroreactivity by state is shown in Figure 3. No significant associations were found for sex or month of sample submission.

When considering each *Bartonella* spp. seroreactivity individually, similar demographic and geographic trends were seen. Seroreactivity for *Bh* was significantly lower in toy (aOR, 0.45; 95% CI, 0.27-0.72), nonsporting (aOR, 0.37, 95% CI, 0.17-0.72), and hound (aOR, 0.49; 95% CI, 0.25-0.89) breeds compared to mixed breeds, and in puppy (aOR, 0.12; 95% CI, 0.01-0.55), junior (aOR, 0.31; 95% CI, 0.11-0.7),

mature (aOR, 0.7; 95% CI, 0.5-0.98), and senior (aOR, 0.71; 95% CI, 0.51-0.97) dogs compared to adult dogs. Seroreactivity for Bh was significantly higher in the West South Central (aOR, 2.04; 95% CI, 1.04-4.07) and South Atlantic (aOR, 2.07; 95% CI, 1.11-3.99) regions compared the Pacific region. No significant associations were found for *Bh* for sex or month of sample submission. Seroreactivity for *Bvb* was significantly lower only in toy (aOR, 0.5; 95% CI, 0.31-0.77) and hound (aOR, 0.55; 95% CI, 0.3-0.95) breeds compared to mixed breeds. Seroreactivity for *Bvb* was significantly higher in the West South Central (aOR, 2.21; 95% CI, 1.15-3.91) and South Atlantic (aOR, 1.89; 95% CI, 1.07-3.42) regions compared the Pacific region. No significant associations were found for *Bvb* for age, sex, or month of sample submission. Seroreactivity for *Bk* was significantly lower in toy (aOR, 0.38; 95% CI, 0.19-0.7), sporting (aOR, 0.56; 95% CI, 0.34-0.91), and hound (aOR, 0.25; 95% CI, 0.08-0.64) breeds compared to mixed breeds, and in mature (aOR, 0.63; 95% CI, 0.41-0.98), and senior (aOR, 0.64; 95% CI, 0.42-0.97) dogs compared to adult dogs. Unlike the other species, *Bk* seroreactivity was not independently associated with census region. Also, no significant associations were found for *Bk* for sex or month of sample submission. Maps showing *Bh*, *Bvb*, and *Bk* seroreactivity by region are shown in Figure 4.

Of the 5957 dogs tested for *Bartonella*, 4166 (70%) had CBC results available and 4331 (73%) had serum biochemistry results available. The proportions of dogs seroreactive to *Bartonella* spp. that had each CBC and serum biochemistry variable result are shown in Tables 2 and 3, respectively.

4 | DISCUSSION

Using whole cell lysates from *Bh*, *Bvb*, and *Bk*, ELISA tests were developed and purposely optimized for 100% analytical specificity, resulting in approximately 40% analytical sensitivity compared to seroreactivity determined by IFA as the reference standard. When nearly 6000 stored serum samples from dogs were tested with these ELISAs, the overall proportion of dogs seroreactive to any *Bartonella* spp. was 6.1%. Seroreactivity for *Bvb* was most common (5%), followed by *Bh* (4.3%) and *Bk* (2.4%). Demographically, a smaller proportion of purebred dogs (5.6%)—specifically toy breeds (3.9%)—compared to mixed breed dogs (7.5%) were *Bartonella* spp. seroreactive. A higher proportion of adult dogs (7.3%) were *Bartonella* spp. seroreactive compared to dogs <1 year of age (3.4%). Geographically, *Bartonella* spp. seroreactivity was variable across US regions, with the highest proportion of dogs seroreactive in the South Atlantic and West South Central census regions (8.8% and 9.8%, respectively). When considering clinicopathologic variables, *Bartonella* seroreactivity was most common in dogs with hyperglobulinemia (ie, 13.5% of dogs with hyperglobulinemia were *Bartonella* spp. seroreactive), neutropenia (13.5%), or increased Ca×P product (12%) compared to dogs with those results within reference intervals. Because causality cannot be proven by a cross-sectional study or by seroreactivity alone, and because other causes of clinicopathologic abnormalities were not investigated, our results do not indicate that any of these clinicopathologic abnormalities occur secondary to bartonellosis.

Demographic and geographic findings for *Bartonella* spp. exposure were broadly comparable to previously reported patterns. Although no difference was found in the proportion of *Bartonella* spp. seroreactivity between male and female dogs, neuter status was not included in the demographic information provided for this study. In previous studies examining *Bartonella* spp. seroreactivity by dog sex, intact dogs had the highest seroprevalence.[34,51] In our study, a higher proportion of mixed breed dogs had *Bartonella* spp. seroreactivity (7.5%) compared to purebred dogs (5.6%), and specifically compared to toy breeds (3.9%). In a seroprevalence study of *Bartonella* spp. exposure (based on IFA) in dogs across North America, mixed breed dogs were more likely to be *Bartonella* spp. seroreactive than purebred dogs.[34] It remains unknown what underlies this risk factor, but increased risk for *Bartonella* spp. exposure in mixed breed dogs may be associated with confounding factors such as lack of flea or tick control, or outdoor lifestyle. Similarly, toy breeds may have lifestyle factors that lead to less exposure to CVBDs, such as spending more time indoors. Interestingly, the low proportion of toy breeds with *Bartonella* spp. seroreactivity was independent of geographic region, suggesting that this finding is not solely an effect of different dog breed ownership trends in different parts of the country. Previous studies have found different proportions of *Bartonella* spp. exposure in dogs from various geographic regions, but are in agreement that exposure can be seen broadly distributed across all geographic regions of North America, and not restricted to certain states or regions.[31,34] Our results confirm this observation, showing even in the lowest risk regions (Pacific, West North Central) over 4% of dogs were *Bartonella* seroreactive.

Because our study was retrospective and based on seroreactivity in a single serum sample, it is not possible to distinguish whether the antibodies detected by ELISA were indicative of recent acute infection, chronic active infection, or previous infection with effective clearance of bacteria (exposure). It is possible that some or all of the seroreactive dogs were previously exposed, but not actively infected with a *Bartonella* spp. Because of this limitation, it is not possible to speculate on potential mechanistic explanations or pathophysiology underlying the clinicopathologic abnormalities associated with *Bartonella* spp. seroreactivity reported here. Hypoalbuminemia and hyperglobulinemia can occur in a range of infectious and non-infectious diseases in dogs, so although these clinicopathologic abnormalities are clinically relevant, they are nonspecific.

To illustrate the differences between groups in this sample of dogs, there were 367 mixed breed dogs from regions with high seroreactivity (West South Central or South Atlantic); of these, 46 were *Bartonella* spp. seroreactive (13%). Among those dogs, 30% with both high globulin and low albumin concentrations and 17% with increased Ca×P product were *Bartonella* spp. seroreactive. In comparison, there were 353 purebred dogs from low seroreactivity regions (West North Central or Pacific) with albumin, globulin, and Ca×P results all within reference range. Of these, only 16 (5%) were *Bartonella* seroreactive. Despite the potential limitations of seroreactivity as an indicator of *Bartonella* exposure, future studies should investigate whether specific clinicopathologic abnormalities are associated with clinical bartonelloses in dogs.

Our study had some limitations based on use of a convenience sample of dog serum samples from a commercial diagnostic laboratory. Because dogs were tested by a national diagnostic laboratory and samples were not collected randomly, selection bias was minimized but not entirely absent. Only dogs that were examined at veterinary clinics and had blood samples submitted for testing were included. Therefore, dogs from remote or lower income areas may have been undersampled, because such dogs may be less likely to receive routine veterinary care. Healthy dogs also may be less likely to have routine blood tests performed than chronically ill dogs, and thus sick dogs may be overrepresented in this sample. Medical and travel history, as well as any illnesses (infectious or otherwise) or exposure to CVBDs, for the dogs making up this sample are unknown, because this information is not routinely provided to the diagnostic laboratory when requesting these tests. Because our study included only results collected by a single laboratory, it is not representative of the overall prevalence of *Bartonella* spp. seroreactivity in all dogs in the United States. However, there is no reason to believe that samples would be submitted preferentially to any particular diagnostic laboratory for reasons related to likelihood of a positive *Bartonella* test, and thus this possibility likely contributes little bias to the sample. Demographic data relied on veterinarian reporting, which may not be accurate in the case of unknown or estimated ages of rescue dogs, or unknown breed or miscategorization of breed in mixed breed dogs or dogs without pedigrees.

Other limitations of our study include limitations of the ELISA test for seroreactivity to *Bartonella* spp. The sensitivity and specificity estimates for the ELISA tests reported here are based on analytical accuracy (ie, how likely each ELISA assay was to detect *Bartonella* spp. antibodies in IFA seroreactive and IFA nonseroreactive samples). In contrast, the epidemiologic and clinical sensitivity and specificity (ie, the performance of this test in populations of dogs with and without bartonellosis) have not been determined. Epidemiologic and clinical sensitivity of this assay are mainly impacted by the biology of, and immune response to, *Bartonella* spp., as well as by sampling strategy and timing. Because the ELISA cutoff was set to maximize specificity, false negative results may have occurred in our study, leading to an underestimate of the true proportion of dogs with *Bartonella* spp. seroreactivity. Also, dogs considered ELISA seroreactive may have had higher IFA *Bartonella* spp. antibody titers, whereas dogs with lower IFA titers would have been considered ELISA nonseroreactive. Generally, the highest *Bartonella* spp. antibody titers are thought to occur in dogs with endocarditis and during acute phase seroconversion, and thus it is possible those conditions would be preferentially detected with this low sensitivity ELISA.[6,11,52] Conversely, because cross-reactivity of the ELISA was only tested for *Rickettsia* spp., cross-reactivity potentially could occur with other bacteria, leading to false positives and an overestimation of the proportion of dogs with *Bartonella* spp. seroreactivity in our study. Currently, IFA serology is the gold standard for determination of exposure to *Bartonella* spp. for both diagnostic and serosurvey purposes. However, this method also has limitations, including low sensitivity for detection of antibodies in dogs with *Bartonella* spp. DNA PCR amplified from blood or tissues.[5,44,53-55] Previous studies have shown poor associations between IFA seroreactivity and bacteremia,[44,53] with IFA antibody reactivity to *Bartonella* species antigens detected in <50% of dogs in which active infection can be documented.[5] Whether such is the case for the 3 ELISA assays used in our study has not yet been determined.

Although *Bartonella* spp. seroreactive dogs were found throughout the US, results of our seroepidemiological study further emphasize the important regional and demographic differences in *Bartonella* spp. seroreactivity in dogs. Future prospective studies are needed to define the clinical relevance of *Bartonella* spp. seroreactivity in dogs.

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CONFLICT OF INTEREST DECLARATION

Edward B. Breitschwerdt is a co-founder, shareholder and Chief Scientific Officer for Galaxy Diagnostics, a company that provides advanced diagnostic testing for the detection of *Bartonella* species infections. He also consults for IDEXX Reference Laboratories. Brendon Thatcher, Ariel Carruth, Anton Mestek, Jesse Buch, Melissa Beall and Ramaswamy Chandrashekar are employees of IDEXX. No other authors have a conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

All samples were acquired with approval from the IDEXX Animal Welfare Committee, and remain the property of IDEXX Laboratories Inc.

HUMAN ETHICS APPROVAL DECLARATION

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

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