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Bartonella Seroepidemiology in Dogs from North America, 2008–2014

E. Lashnits, M. Correa, B.C. Hegarty, A. Birkenheuer, and E.B. Breitschwerdt

Background: Improved understanding of *Bartonella* species seroepidemiology in dogs may aid clinical decision making and enhance current understanding of naturally occurring arthropod vector transmission of this pathogen.

Objectives: To identify demographic groups in which *Bartonella* exposure may be more likely, describe spatiotemporal variations in *Bartonella* seroreactivity, and examine co-exposures to other canine vector-borne diseases (CVBD).

Animals: A total of 15,451 serology specimens from dogs in North America were submitted to the North Carolina State University, College of Veterinary Medicine Vector Borne Disease Diagnostic Laboratory between January 1, 2008, and December 31, 2014.

Methods: *Bartonella henselae*, *Bartonella koehlerae,* and *Bartonella vinsonii* subspecies *berkhoffii* indirect fluorescent antibody (IFA) serology results, as well as results from a commercial assay kit screening for *Dirofilaria immitis* antigen and *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi* antibodies, and *Ehrlichia canis*, *Babesia canis*, *Babesia gibsoni*, and *Rickettsia* species IFA results were reviewed retrospectively.

Results: Overall, 3.26% of dogs were *Bartonella* spp. seroreactive; *B. henselae* (2.13%) and *B. koehlerae* (2.39%) were detected more frequently than *B. vinsonii* subsp. *berkhoffii* (1.42%, $P < 0.0001$). Intact males had higher seroreactivity (5.04%) than neutered males (2.87%, $P < 0.0001$) or intact or spayed females (3.22%, $P = 0.0003$). Mixed breed dogs had higher seroreactivity (4.45%) than purebred dogs (3.02%, $P = 0.0002$). There was no trend in seasonal seroreactivity; geographic patterns supported broad distribution of exposure, and co-exposure with other CVBD was common.

Conclusions and Clinical Importance: *Bartonella* spp. exposure was documented throughout North America and at any time of year. Male intact dogs, mixed breed dogs, and dogs exposed to other CVBD have higher seroreactivity to multiple *Bartonella* species.

Key words: Canine; Seroreactivity; Zoonoses.

Members of the genus *Bartonella*, fastidious gram-negative rod-shaped hemotropic and endotheliotropic bacteria, are important emerging pathogens in dogs and humans worldwide.[1–3] For the past 2 decades, an increasingly diverse number of *Bartonella* species have been isolated or detected using PCR in a wide range of animals including cats, dogs, and humans, as well as many wildlife reservoir and arthropod vector species.[4] *Bartonella* persists in erythrocytes and vascular endothelial cells, causing chronic relapsing bacteremia.[2,5–8]

Abbreviations:

CI	confidence interval
CVBD	canine vector-borne diseases
IFA	immunofluorescent antibody
OR	odds ratio
VBDDL	Vector Borne Disease Diagnostic Laboratory

Worldwide, domestic dogs can be infected with at least 10 *Bartonella* species.[3,9] *Bartonella vinsonii* subsp. *berkhoffii*, *B. henselae*, and *B. koehlerae* represent the most frequent species found infecting dogs in North America.[10] All 3 of these species have been implicated as pathogenic in cases of endocarditis in dogs[7,11–13] and have been associated with other clinical abnormalities in dogs including vasoproliferative diseases, vasculitis, myocarditis, polyarthritis, granulomatous disease (lymphadenitis, rhinitis, hepatitis), epistaxis, and neurologic diseases.[14–23] However, because they are emerging pathogens in dogs, the spectrum of diseases associated with *Bartonella* infection has not been fully elucidated.

Bartonella species are primarily arthropod vector transmitted.[4,24,25] A wide variety of *Bartonella* species have coevolved with their specific vertebrate reservoirs hosts, among which transmission occurs via the arthropod vectors that typically infest these reservoirs (eg, cats are the primary reservoir host for *B. henselae* and *B. henselae* is transmitted between cats by the cat flea *Ctenocephalides felis*).[2,4,24] To date, no definitive vector has been identified for natural transmission of *Bartonella* to dogs. However, on the basis of case reports,[4,19,26–28] serosurveys,[29–36] surveys of arthropod vectors,[37–41] and experimental data (Lappin and Breitschwerdt, unpublished data),[42–44] ticks (including *Ixodes*

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spp. and *Rhipicephalus sanguineus*), and fleas (*C. felis* and *Ctenocephalides canis*) have been proposed as potential vectors for *Bartonella* spp. transmission in dogs.

To date, a limited number of *Bartonella* seroepidemiologic studies have been performed involving large numbers of dogs from different regions of North America. *Bartonella* seroepidemiologic studies can provide important information about temporospatial distribution, disease prevalence, and potentially may help elucidate modes of transmission. Regional and seasonal differences in *Bartonella* spp. seroreactivity, as well as associations with other vector-borne pathogens across dog populations, can indirectly implicate potential arthropod vectors. In addition, seroreactivity data can guide clinical decision making. For example, coinfection with multiple vector-borne pathogens can cause more severe manifestations of disease, and determining exposure to *Bartonella* in dogs suspected of other CVBD is warranted.[45,46]

To better understand the epidemiology and distribution of *Bartonella* infection in dogs in North America, we analyzed a large diagnostic laboratory database. The purpose of our study was to identify *Bartonella* seroreactivity differences among demographic groups, describe variations in temporal and geographic patterns of *Bartonella* seroreactivity, and examine co-exposure between *Bartonella* and other vector-borne pathogens. Improved understanding of seroepidemiologic patterns may aid clinical decision making, as well as increase our understanding of transmission by arthropod vectors in naturally infected dogs.

Materials and Methods

Canine serum samples submitted to the North Carolina State University, College of Veterinary Medicine, Vector Borne Disease Diagnostic Laboratory (VBDDL), over a 7-year period between January 1, 2008, and December 31, 2014, were selected for study. Samples originated from veterinary hospitals in North America for diagnostic immunofluorescent antibody (IFA) testing for *Bartonella* and other vector-borne diseases. Available patient information included date of sample collection, date of sample receipt, signalment, and veterinary practice location. Test results were retrospectively reviewed, and the extracted data were analyzed. This a convenience sample given that the NCSU VBDDL is 1 of several laboratories where canine *Bartonella* serology samples can be submitted in North America. Samples were excluded if a sample from the same dog was submitted within the prior 5 weeks, to exclude convalescent samples.

Serum samples included in the study were submitted by the attending clinician to the VBDDL for individual serologic tests for ≥ 1 *Bartonella* spp., or for a comprehensive vector-borne pathogen serology panel. The VBDDL is not informed as to the motivation for testing, and thus, this information was not available in the data. Between January 2008 and July 2011, only *B. henselae* and *B. vinsonii* subsp. *berkhoffii* were used as antigens for IFA testing. After July 2011, the serology panel was amended to include *B. koehlerae*. Before July 2012, comprehensive panels included a SNAP 4Dx; starting in July 2012, this was changed to a SNAP 4Dx PLUS[a] test. Other antigens included in comprehensive serology panels for dogs included *Ehrlichia canis*, *Babesia canis*, *Babesia gibsoni*, and *Rickettsia* species. A subset of samples also was submitted for *Bartonella* alpha proteobacteria growth medium (BAPGM) culture enrichment and polymerase chain reaction, performed as previously described.[47]

All IFA antigens were grown in vitro at the VBDDL. *Bartonella* strains were isolated from naturally infected cats or dogs with species characterizations made using PCR amplification and DNA sequence analysis techniques. A canine isolate of *B. vinsonii* subsp. *berkhoffii* genotype I (NCSU 93CO-01, ATCC type strain #51672) and feline isolates of *B. henselae* H-1 strain (NCSU 93FO-23) and *B. koehlerae* (NCSU 09FO-01) were passed from agar plate grown cultures into a *Bartonella*-permissive cell line, DH82 cells (a canine monocytoid cell line) to obtain antigens for IFA testing; the same isolates were used across all years of this study (2008–2014). For each antigen, heavily infected cell cultures were spotted onto 30-well teflon-coated slides, air-dried, acetone fixed, and stored frozen. Serum samples diluted in phosphate-buffered saline solution containing normal goat serum, Tween-20, and powdered nonfat dry milk to block nonspecific antigen binding sites were screened at dilutions of 1:16 to 1:64. All sera that were reactive at a titer of 1:64 were further tested with 2-fold dilutions out to 1:8,192. Fluorescein-conjugated goat anti-dog IgG was used to visualize bacteria within cells using a fluorescent microscope. To avoid confusion with possible nonspecific binding found at low dilutions, a cutoff of 1:64 was used to define a seroreactive titer.

Regions were based on address provided with sample submission and defined by US census region as follows: Pacific—WA, OR, CA; Mountain—ID, NV, MT, WY, UT, CO, AZ, NM; West North Central—ND, SD, NE, KS, MN, IA, MO; West South Central—OK, AR, TX, LA; East North Central—WI, IL, IN, OH, MI; East South Central—KY, TN, MS, AL; South Atlantic —MD, DE, WV, VA, NC, SC, GA, FL; Middle Atlantic—NY, PA, NJ; New England—ME, NH, VT, MA, CT. Dogs from AK and HI (n = 8) were not included in these regions. Canada was considered as 1 region. Breed groups were defined using AKC breeds; breeds that are not considered by the AKC were grouped with mixed breed dogs. Seasonality was based on month: autumn: September, October, and November; winter: December, January, and February; spring: March, April, and May and; summer: June, July, and August.

Descriptive statistics were obtained, and seroreactivity to each *Bartonella* species was compared for different demographic, regional, and chronologic variables using the chi-square test. Logistic regression was used to identify univariate associations between *Bartonella* seroreactivity and selected comparison groups. Possible effects on the odds ratios (ORs) of the low event per variable were checked using the Firth adjustment, also known as the penalization approach.[48] ORs and 95% confidence intervals (CIs) for the ORs were estimated. Maps were created using ArcGIS.[b] Boundaries were created from publicly available data from the US Census Bureau[49] and Statistics Canada,[50] using the North American Datum (NAD) 1983 geographic coordinate system with Geodetic Reference System (GRS) 1980 spheroid. For each *Bartonella* spp., the minimum number of samples needed to detect a single positive sample was calculated based on the overall seroreactivity for that species in North America. States were excluded from seroreactivity maps if the number of samples submitted from a state was lower than the minimum number calculated above. Data analysis was performed using SAS/STAT software[c] and OpenEpi.[d] Statistical significance was considered at a P value of ≤0.05.

Results

Over 7 years, from 2008 through 2014, 15,451 individual canine serum samples from 15,295 dogs were submitted to the VBDDL for *Bartonella* IFA serology as previously described. Of these, 14,935 dogs (96.7%) were tested for both *B. henselae* and *B. vinsonii* subsp.

berkhoffii antibodies; 4,517 dogs (29.2%) were tested for *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, and *B. koehlerae* antibodies. The highest number of samples originated from the South Atlantic region (6,548, 42.4%); the fewest samples came from the New England region (367, 2.4%). The region was not reported for 13 samples (0.08%). The largest number of samples was submitted in 2009 (2,581, 16.7%) and the smallest number in 2012 (1,780, 11.5%). The breeds most frequently represented in the study population included mixed breed dogs (2,608, 16.9%), Labrador Retrievers (1,603, 10.4%), and Golden Retrievers (858, 5.5%), with dogs from each remaining breed (188 breeds) making up <5% of the study population. Breed was not reported for 1 sample. Ages ranged from 4 weeks to 20 years, with a median of 6.0 years; the age was not reported for 642 dogs. There were 7,482 males (5,855 neutered, 78%) and 7,691 females (6,752 spayed, 88%). Sex was not reported for 278 samples (1.8%). Breed, sex, region, date of submissions, and seroreactivity are summarized in Table 1.

On the basis of IFA seroreactivity, 504 (3.26%) dogs were seroreactive to ≥1 *Bartonella* spp. Seroreactivity to *B. henselae* (2.13%) and *B. koehlerae* (2.39%) antigens was detected more frequently than seroreactivity to *B. vinsonii* subsp. *berkhoffii* (1.42%, $P < 0.0001$) antigen (Fig 1).

The youngest seroreactive dog was 4 weeks of age, and the oldest was 20 years of age, with a median age of 6 years. The median age for both seropositive and seronegative dogs was 6.0 years.

There was no statistically significant difference in overall seroreactivity based upon sex (248 seroreactive females and 250 seroreactive males). However, intact male dogs were more likely to be seroreactive (5.04%) than neutered males (2.87%; OR, 1.80; 95% CI, 1.37–2.35) or intact or spayed females (3.22%; OR, 1.59; 95% CI, 1.23–2.05; also see Table 2). When the

Table 1. Summary of samples submitted for *Bartonella* serology and number seroreactive to each antigen.

	Bh Tested	*Bh*+	% *Bh*+	*Bvb* Tested	*Bvb*+	% *Bvb*+	*Bk* Tested	*Bk* +	% *Bk* +	Any spp. Tested	% Of Total
All	15,017	320	2.1	15,365	218	1.4	4,521	108	2.4	15,451	—
Sex											
F	893	19	2.1	931	12	1.3	257	7	2.7	939	6.1
FS	6,597	144	2.2	6,715	90	1.3	1,999	49	2.5	6,752	43.7
M	1,556	47	3.0	1,612	45	2.8	398	9	2.3	1,627	10.5
MC	5,702	106	1.9	5,829	68	1.2	1,746	40	2.3	5,855	37.9
Breed											
Herding	1,747	39	2.2	1,784	38	2.1	518	15	2.9	1,797	11.6
Hound	1,663	41	2.5	1,697	25	1.5	483	8	1.7	1,714	11.1
Mixed	2,533	80	3.2	2,593	52	2.0	784	27	3.4	2,608	16.9
Nonsporting	1,035	20	1.9	1,060	13	1.2	305	2	0.7	1,067	6.9
Sporting	3,568	61	1.7	3,649	33	0.9	1,003	23	2.3	3,660	23.7
Terrier	1,245	19	1.5	1,270	15	1.2	420	14	3.3	1,276	8.3
Toy	1,541	24	1.6	1,581	9	0.6	494	6	1.2	1,591	10.3
Working	1,684	36	2.1	1,730	33	1.9	514	13	2.5	1,737	11.2
Region											
Canada	465	11	2.4	469	3	0.6	64	1	1.6	472	3.1
E. N. Central	2,051	42	2.0	2,059	19	0.9	489	8	1.6	2,063	13.4
E. S. Central	532	10	1.9	541	6	1.1	212	5	2.4	544	3.5
Mid-Atlantic	1,067	20	1.9	1,155	19	1.6	398	6	1.5	1,164	7.5
Mountain	627	10	1.6	657	12	1.8	129	5	3.9	661	4.3
New England	356	14	3.9	363	6	1.7	152	5	3.3	367	2.4
Pacific	521	15	2.9	612	11	1.8	210	5	2.4	619	4.0
S. Atlantic	6,421	116	1.8	6,508	77	1.2	1,827	37	2.0	6,548	42.4
W. N. Central	477	11	2.3	487	5	1.0	189	7	3.7	494	3.2
W. S. Central	2,487	71	2.9	2,501	60	2.4	844	29	3.4	2,506	16.2
Year											
2008	2,456	138	5.6	2,506	53	2.1	—	—	—	2,516	16.3
2009	2,460	50	2.0	2,556	52	2.0	—	—	—	2,581	16.7
2010	2,029	33	1.6	2,111	35	1.7	—	—	—	2,140	13.9
2011	1,987	13	0.7	2,064	18	0.9	51	1	2.0	2,076	13.4
2012	1,729	14	0.8	1,771	14	0.8	117	14	12.0	1,780	11.5
2013	1,920	29	1.5	1,921	20	1.0	1,917	60	3.1	1,921	12.4
2014	2,436	43	1.8	2,436	26	1.1	2,436	33	1.4	2,437	15.8
Month											
December–February	3,454	76	2.2	3,533	65	1.8	982	34	3.5	3,554	23.0
March–May	3,829	79	2.1	3,921	47	1.2	1,082	25	2.3	3,940	25.5
June–August	3,994	83	2.1	4,072	59	1.4	1,221	24	2.0	4,096	26.5
September–November	3,740	82	2.2	3,839	47	1.2	1,236	25	2.0	3,861	25.0

Bh, *B. henselae*; *Bvb*, *B. vinsonii* subsp. *berkhoffii*; *Bk*, *B. koehlerae*; Any, seroreactive to any 1 or more *Bartonella* spp.

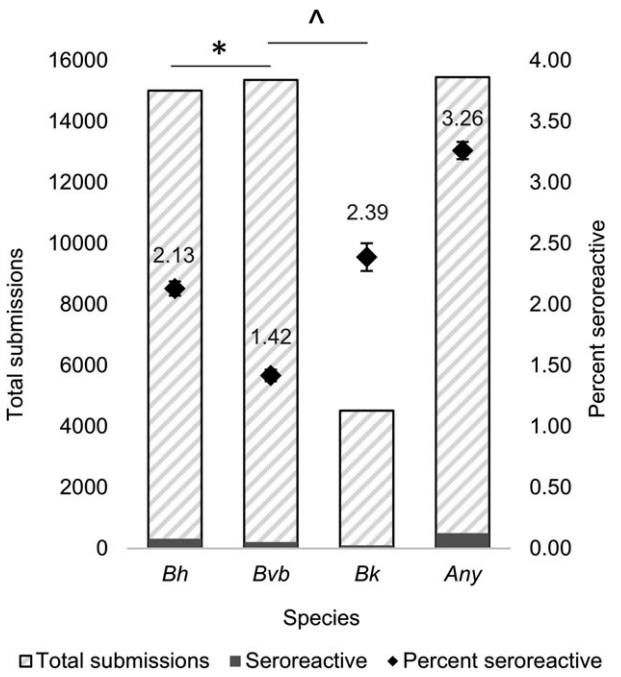

Fig. 1. *Bartonella* spp. seroreactive dogs, 2008–2014. *Bh*, *B. henselae*; *Bvb*, *B. vinsonii* subsp. *berkhoffii*; *Bk*, *B. koehlerae*; Any, positive to any one or more species. Numbers represent the percent of dogs seroreactive to each *Bartonella* species (right side scale). Error bars represent standard error for the percent of dogs seroreactive to each *Bartonella* species. Statistically significant differences ($P \leq 0.05$) between percent of dogs seroreactive to each species represented by * and ^.

proportion of dogs seroreactive to each species of *Bartonella* was determined using 2 × 2 tables, male intact dogs had higher seroreactivity than male neutered dogs or female intact or spayed dogs for both *B. henselae* and *B. vinsonii* subsp. *berkhoffii*, but not for *B. koehlerae*. There was no difference in seroreactivity between female intact and female spayed dogs, either in overall seroreactivity or when analyzed for each individual *Bartonella* species.

Mixed or non-AKC breed dogs were more likely to be seroreactive to any *Bartonella* spp. (4.45%) than were purebred dogs (3.02%; OR, 1.49; 95% CI, 1.21–1.85). When compared to mixed breed dogs, multiple categories of pure breed dogs were less likely to be *Bartonella* spp. seroreactive (Table 2). The actual ORs are presented in Table 2, given the negligible differences using the maximum likelihood estimates with logistic regression and logistic regression with the Firth bias reduction for the possible effect of low event per variable.

Overall proportions of seroreactive dogs by region are shown in Figure 2. For any *Bartonella* species, the highest proportions of seroreactive dogs in the study population were found in the New England, Pacific, and West South Central regions (5.18, 4.52, and 4.39%, respectively), whereas the lowest seroreactivity was found in the South Atlantic and East South Central regions (2.75 and 2.76%). *Bartonella henselae* had the highest proportion of seroreactive dogs in the New England region (3.93%), and lowest in the Mountain region

Table 2. Odds ratios for main effects based on logistic regression for seroreactivity to any of the 3 *Bartonella* spp. tested.

	OR	95% CI	*P* Value
Sex			
Versus MI			
F	0.64	0.42–0.98	0.0409*
FS	0.62	0.47–0.81	0.0004*
MC	0.55	0.42–0.73	<0.0001*
Breed			
Versus mixed			
Herding	0.78	0.5–1.06	0.1101
Hound	0.77	0.56–1.06	0.1082
Nonsporting	0.56	0.37–0.85	0.0065*
Sporting	0.53	0.40–0.70	<0.0001*
Terrier	0.64	0.44–0.93	0.0197*
Toy	0.45	0.30–0.66	<0.0001*
Working	0.74	0.54–1.02	0.0653
Region			
Versus S. Atlantic			
Canada	1.19	0.69–2.03	0.7554
E. N. Central	1.05	0.78–1.41	0.1350
E. S. Central	0.95	0.55–1.66	0.2416
Mid-Atlantic	1.11	0.77–1.60	0.3798
Mountain	1.27	0.81–2.00	0.9722
New England	2.03	1.24–3.30	0.0381*
Pacific	1.66	1.10–2.49	0.1662
W. N. Central	1.31	0.79–2.18	0.9145
W. S. Central	1.62	1.26–2.06	0.0338*

Season of submission did not contribute significantly to the model.

P values were obtained using analysis of maximum likelihood estimates and Wald chi-square test. Statistical significance indicated by * at $P \leq 0.05$.

(1.59%). *Bartonella vinsonii* subsp. *berkhoffii* had the highest proportion of seroreactive dogs in the West South Central region (2.4%) and lowest in Canada and East North Central regions (0.64 and 0.92%). *Bartonella koehlerae* had the highest proportion of seroreactive dogs in the Mountain and West North Central regions (3.88 and 3.7%) and lowest in the Middle Atlantic, Canada, and East North Central regions (1.51, 1.56, and 1.64%, respectively). Based on logistic regression, region was a significant factor for seroreactivity against any *Bartonella* spp. ($P = 0.0036$). With this model, dogs from the New England, Pacific, and West South Central regions were more likely than dogs from the South Atlantic region to be seroreactive against any of the 3 *Bartonella* spp. antigens (Table 2).

Seroreactivity varied by state and species (Fig 3). When states with low numbers of submissions were removed, state-by-state percentage seroreactive for *B. henselae* ranged from 0% (NM, 0/71) to 6.67% (Washington, 4/60), for *B. vinsonii* subsp. *berkhoffii* ranged from 0% (NM, 0/71 and IN, 0/300) to 3.8% (OK, 3/79), and for *B. koehlerae* ranged from 0% (VA, 0/171) to 6.59% (MO, 6/91).

Overall seroreactivity varied by year (Fig 4), with the highest overall seroreactivity in 2008 (6.92%), and lowest in 2011 (1.2%; OR, 6.095; 95% CI, 3.991–9.308). Seroreactivity was particularly high for *B. henselae* in 2008

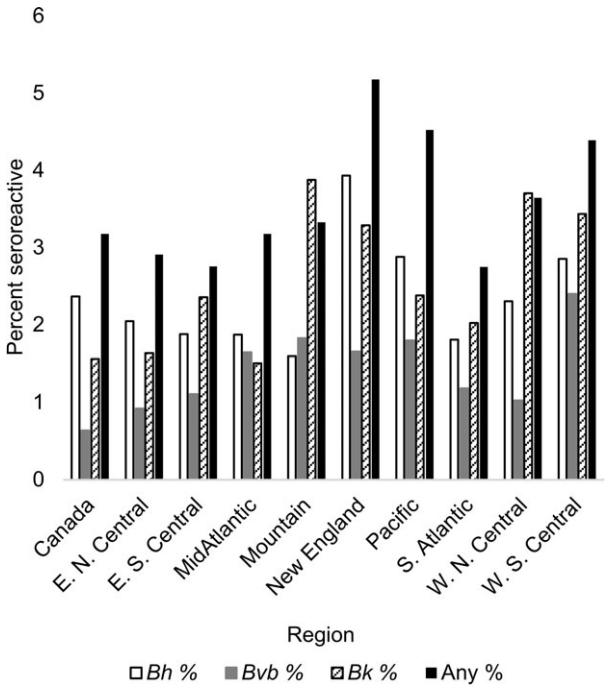

Fig. 2. *Bartonella* spp. seroreactivity by region. *Bh*, *B. henselae*; *Bvb*, *B. vinsonii* subsp. *berkhoffii*; *Bk*, *B. koehlerae*; Any, positive to any one or more species.

(5.62%) compared to 2011 and 2012 (0.65 and 0.81%), and only increased slightly again in 2013 and 2014 (1.51 and 1.77%). Similarly, *B. vinsonii* subsp. *berkhoffii* seroreactivity was highest in 2008 (2.11%), decreased to its lowest in 2011 and 2012 (0.87 and 0.79%), and increased slightly again in 2013 and 2014 (1.04 and 1.07%). *Bartonella koehlerae* serology was not offered before July 2011, but the highest annual seroreactive rate for *B. koehlerae* was in 2012 (11.97%), before it too decreased in 2013 and 2014 (3.13 and 1.35%). There was no significant trend in seroreactivity by month and no seasonal trend either for overall seroreactivity or seroreactivity to each of the *Bartonella* spp. (Fig 4). The highest overall seroreactivity was in June (4.85%) and the lowest in July (1.82%). Season did not contribute significantly to the logistic regression model.

Of dogs tested for *Bartonella*, 13,803 also had concomitant SNAP 4Dx or SNAP 4Dx PLUS testing performed, indicating 2.12% positive for *Anaplasma platys*/*phagocytophilum*, 4.59% positive for *B. burgdorferi*, and 5.36% positive for *E. canis*/*ewingii*. Odds ratios for coinfection between *Bartonella* and other vector-borne pathogens are presented in Table 3. Dogs that were *B. henselae* seroreactive had increased risk of being *E. canis* (by IFA), *E. canis*/*E. ewingii* (by SNAP test), *B. burgdorferi*, *A. platys*, *A. phagocytophilum*, *B. canis*, and *Rickettsia* spp. seroreactive. Dogs that were

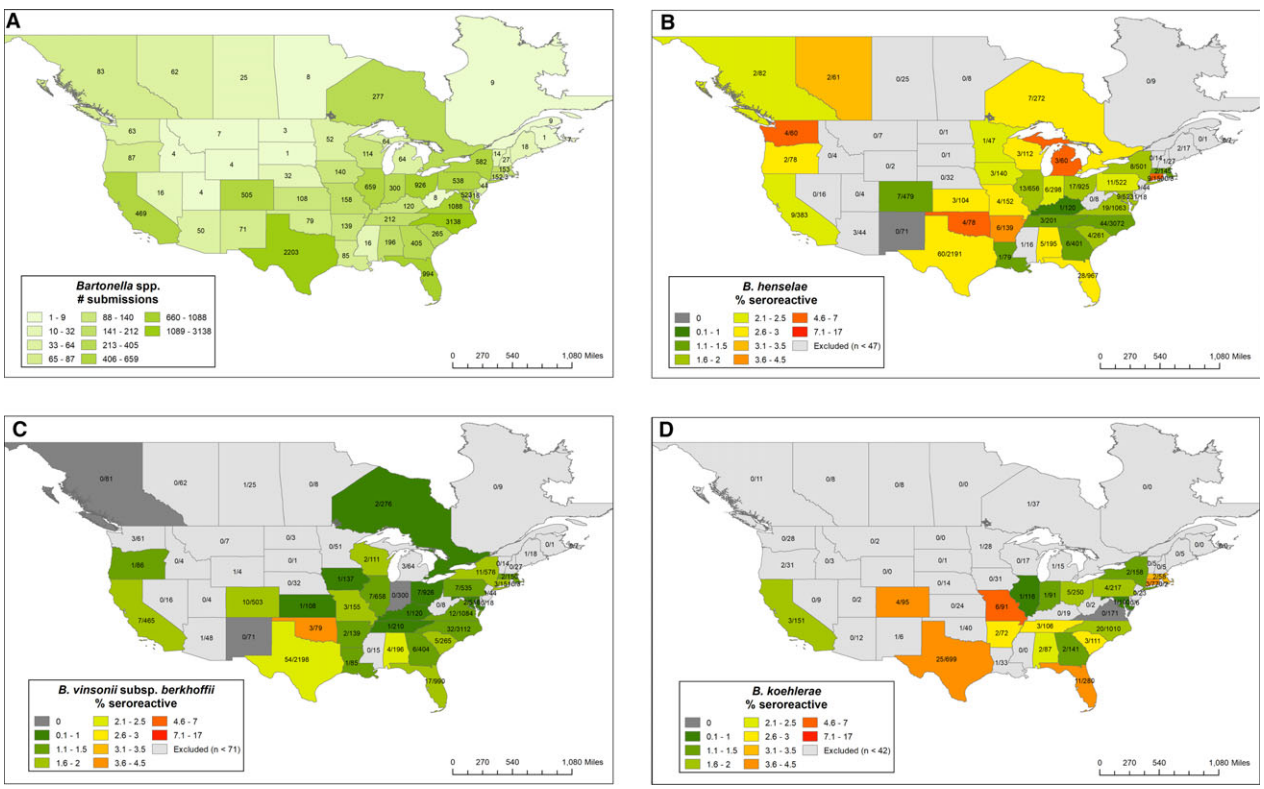

Fig. 3. (**A**) Map showing the total number of samples per state/province submitted for *Bartonella* spp. serology during the study period (2008–2014). (**B–D**) Maps of *Bartonella* spp. seroreactivity in North America. Colors depict the percent of dogs seroreactive for each species, ratios shown within each state or province show number of positive samples in the numerator and total number of samples in the denominator; states with low sample sizes are excluded (shown in gray). Alaska, Hawaii, and Canadian provinces for which no samples were submitted are not shown. (**B**) *B. henselae* seroreactivity. (**C**) *B. vinsonii* subsp. *berkhoffii* seroreactivity. (**D**) *B. koehlerae* seroreactivity.

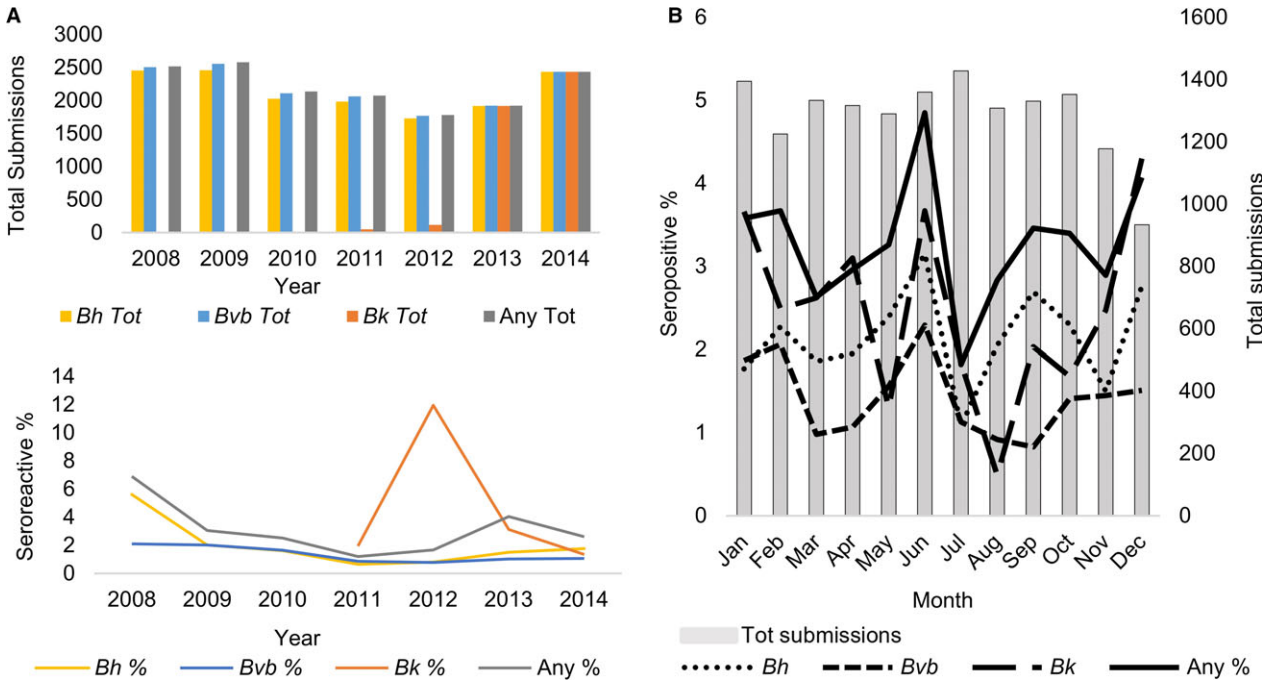

Fig. 4. Annual and monthly trends in *Bartonella* spp. seroreactivity. *Bh*, *B. henselae*; *Bvb*, *B. vinsonii* subsp. *berkhoffii*; *Bk*, *B. koehlerae*; Any, positive to any one or more species. (**A**) Trends by year. Top panel shows total submissions by year; bottom panel shows percent seroreactive by year. *B. koehlerae* was added to the comprehensive serology panel in July 2011. (**B**) Trends by month.

B. vinsonii berkhoffii seroreactive had increased risk of being *E. canis* (by IFA), *E. canis*/*E. ewingii* (by SNAP test), *B. burgdorferi*, *Dirofilaria immitis*, *B. canis*, and *Rickettsia* spp. seroreactive. Dogs that were *B. koehlerae* seroreactive had increased risk of being *E. canis* (by IFA), *E. canis*/*E. ewingii* (by SNAP test), *D. immitis*, and *Rickettsia* spp. seroreactive. All 34 *B. gibsoni* seroreactive dogs were *Bartonella* spp. seronegative.

Coinfections with different *Bartonella* spp. were common (Fig 5). Of 4,517 dogs tested for all 3 *Bartonella* spp., 159 (3.52%) were seroreactive to ≥1 species. The majority of these seroreactive dogs was seroreactive to *B. koehlerae* alone (67/159, 42%) or *B. henselae* alone (33/159, 21%), but 23 (14%) were seroreactive to all 3 *Bartonella* spp. antigens. Very few dogs were seroreactive to *B. vinsonii* subsp. *berkhoffii* alone (7/159, 4%). Dogs that were *B. vinsonii* subsp. *berkhoffii* or *B. koehlerae* seroreactive had an increased likelihood of being *Bartonella* PCR or *Bartonella* alpha proteobacteria growth medium (BAPGM) culture positive compared to dogs seronegative for those *Bartonella* spp. (OR, 5.72; 95% CI, 1.67–19.60; $P = 0.0017$ and OR, 18.69; 95% CI, 5.65–61.86; $P < 0.0001$, respectively). However, *B. henselae* seroreactive dogs were no more likely than *B. henselae* seronegative dogs to be *Bartonella* PCR or BAPGM culture positive (OR, 2.44; 95% CI, 0.57–10.45; $P = 0.2139$).

Discussion

Overall, 3.26% of dogs in our study were *Bartonella* spp. seroreactive, a percentage that is comparable to seroreactivity patterns for other CVBDs among US canine population-wide serosurveys.[5,34,51,52] For comparison, based on the Companion Animal Parasite Council publicly available data for 2014 (the final year of our study), the seroprevalence for the contiguous United States, of *B. burgdorferi*, ehrlichiosis, and anaplasmosis was 6.35, 3.01, and 2.97%, respectively (https://www.capcvet.org/parasite-prevalence-maps). Seroreactivity to *B. henselae* (2.13%) or *B. koehlerae* (2.39%) antigen was detected significantly more frequently than seroreactivity to *B. vinsonii* subsp. *berkhoffii* (1.42%) antigen. Although it was previously thought that *B. vinsonii* subsp. *berkhoffii* was the most common *Bartonella* to infect dogs, recent evidence from 2 studies,[9,30] as well as the results presented here, refutes that assumption.

In our study, male intact dogs had significantly higher seroreactivity (5.04%) than either female dogs (3.22%) or male neutered dogs (2.87%). Male intact status previously has been reported as a high risk category for heartworm disease in dogs.[5,53,54] Mechanistically, lifestyle or socioeconomic factors, rather than a biologic phenomenon, is considered the most likely reason for male intact status as a marker of heartworm disease risk. Additionally, mixed or non-AKC registered breed dogs were more likely to be *Bartonella* spp. seroreactive (4.45%) than purebred dogs (3.02%). It is unknown what underlies either of these risk factors for *Bartonella* infection, and further studies are warranted to investigate confounding factors.

Geographic patterns of seroreactivity did not correspond with other regional CVBD patterns (https://www.capcvet.org/parasite-prevalence-maps). In contrast to previous studies,[30,32] no *Bartonella* species was found

Table 3. Co-exposure between *Bartonella* spp. and other CVBD pathogens.

	OR	95% CI	*P* Value
B. henselae			
Lyme SNAP	2.44	1.59–3.76	<0.0001*
Anaplasma SNAP	2.58	1.42–4.66	0.0012*
Ehrlichia SNAP	1.68	1.05–2.67	0.0277*
E. canis IFA	3.34	2.31–4.85	<0.0001*
Babesia canis IFA	3.93	1.70–9.06	0.0005*
Rickettsia IFA	4.38	3.23–5.93	<0.0001*
HW SNAP	1.36	0.33–5.55	0.6694
B. vinsonii subsp. *Berkhoffii*			
Lyme SNAP	2.42	1.36–4.33	0.002*
Anaplasma SNAP	1.52	0.56–4.15	0.4067
Ehrlichia SNAP	2.79	1.67–4.68	<0.0001*
E. canis IFA	6.00	3.96–9.10	<0.0001*
Babesia canis IFA	5.94	2.37–14.86	<0.0001*
Rickettsia IFA	5.78	3.95–8.47	<0.0001*
HW SNAP	3.90	1.22–12.50	0.0135*
B. koehlerae			
Lyme SNAP	1.95	0.83–4.55	0.1171
Anaplasma SNAP	2.44	0.87–6.84	0.0787
Ehrlichia SNAP	2.33	1.27–4.27	0.0052*
E. canis IFA	3.45	1.84–6.50	<0.0001*
Babesia canis IFA	2.39	0.57–10.05	0.2196
Rickettsia IFA	2.72	1.57–4.71	0.0002*
HW SNAP	7.62	1.70–34.12	0.0018*
Any *Bartonella* spp.			
Lyme SNAP	2.42	1.69–3.46	<0.0001*
Anaplasma SNAP	2.00	1.16–3.46	0.0115*
Ehrlichia SNAP	1.97	1.37–2.83	0.0002*
E. canis IFA	3.31	2.43–4.51	<0.0001*
Babesia canis IFA	3.50	1.68–7.26	0.0003*
Rickettsia IFA	4.34	3.37–5.59	<0.0001*
HW SNAP	3.41	1.56–7.44	0.001*

OR, odds ratio.

ORs represent odds of seroreactivity to each CVBD for sample seroreactive to each *Bartonella* species antigen (or any *Bartonella* spp.), compared to samples not seroreactive to each *Bartonella* antigen (or any *Bartonella* spp.). ORs obtained using Cochran-Mantel-Haenszel test for categorical data.

Statistical significance indicated by * at $P \leq 0.05$.

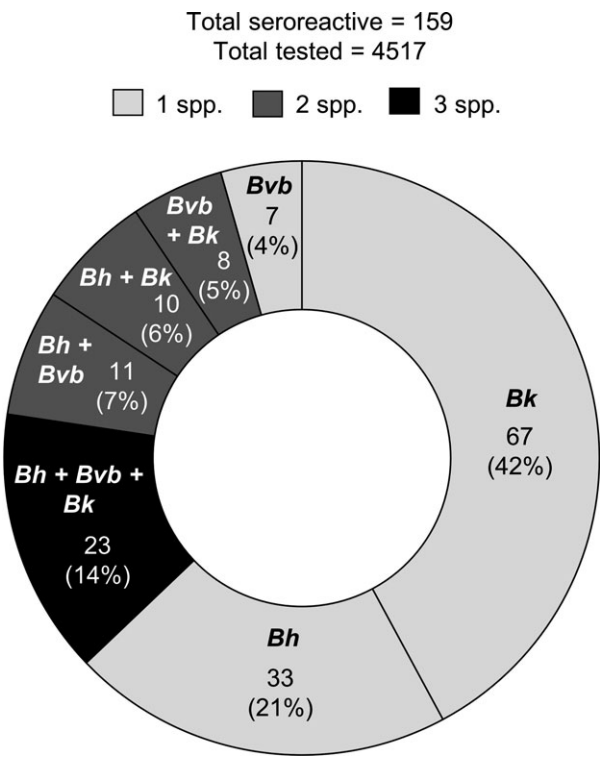

Fig. 5. Coinfection between *Bartonella* spp. *Bh*, *B. henselae*; *Bvb*, *B. vinsonii* subsp. *berkhoffii*; *Bk*, *B. koehlerae*. Numbers within each section show the number of dogs seroreactive to the particular combination of *Bartonella* species represented; percentages in parentheses show the proportion of dogs tested for all 3 *Bartonella* species that were seroreactive to that particular combination of species. Shades show the number of different species to which a dog was seroreactive.

to be most common in dogs from the Southeast or in warmer climates. Rather, seroreactivity was distributed broadly across the North American regions from which samples originated. The largest number of samples originated from the South Atlantic region (42% of samples), which was expected because of the location of the VBDDL in North Carolina. Extrapolations to underrepresented regions (Canada, Mountain and Pacific regions, New England, and areas of the Midwest) should be done with caution given the lower sample numbers from these regions (300–700 samples per region; see Table 1). However, even when excluding states with low sample numbers, there were states with apparently higher exposure that were different for each *Bartonella* species, including *B. henselae* in WA (4/56 seroreactive) and CT (9/141 seroreactive), and *B. koehlerae* in MO (6/91 seroreactive). Because of this finding, it appears important to evaluate each *Bartonella* spp. separately based on their disparate geographic patterns. Future studies using multivariate analysis or statistical modeling could integrate climate and land-use data to identify possible locations with higher *Bartonella* exposure. Clinicians should be aware that *Bartonella* infections in dogs can be seen throughout North America.

No seasonal trend in seroreactivity was found, with seroreactivity varying with no discernable pattern throughout the year. The lack of seasonality may reflect transmission by different vectors at various time points throughout the year, variability among individual dogs in the time required to seroconvert, the duration of infection at the time of testing, or other factors that were not examined in our study. However, if there is no seasonal trend for dogs acquiring *Bartonella* infection, exposure to ≥1 vector is equally likely to occur year-round. Clinicians should be aware that it is possible to detect *Bartonella* seroreactive dogs in North America during any season of the year.

The high risk for co-exposure with *Bartonella* and other vector-borne pathogens has been reported previously[14,26,27,31,33–36] and is consistent with the results of our study. In conjunction with male intact status, sequential or concurrent infection with another vector-borne pathogen may be a marker for lifestyle behaviors that influence a dog's risk of *Bartonella* exposure, including failure to effectively administer flea and

tick prevention products, outdoor exposure, ability to roam, and increased contact with reservoir hosts (eg, feral cats, wild canids such as coyotes, or their ectoparasites).[5,31,55,56] Co-exposure or coinfection with known tick-borne pathogens continues to support ticks as possible vectors for *Bartonella* transmission. As significant rates of coinfection were found for all *Bartonella* spp., and particularly for *B. vinsonii* subsp. *berkhoffii* and *B. henselae*, our data do not specifically implicate any single vector, but provide supportive evidence for many previously proposed vectors including *Rhipicephalus sanguineous*,[26,27,31,35,39,44] *Ixodes* spp,[28,33,36–38,40–43] *Dermacentor variabilis*/*andersonii*,[14,30,31,34,38] or *Amblyomma americanum*.[31,35] However, given the likelihood of CVBD co-exposures and coinfections,[57] screening for *Bartonella* infection should be considered in dogs infected with, or exposed to, other CVBD pathogens. This is particularly important in sick dogs, because treatment with doxycycline, which is indicated for several other vector-borne diseases, does not appear to be effective in eliminating *Bartonella* infection.[58] Thus, doxycycline treatment failure could lead to chronic illness or incomplete resolution of clinical signs or clinicopathologic abnormalities.[23,27,59]

Interestingly, *B. koehlerae* seroreactivity, unlike seroreactivity to *B. henselae* or *B. vinsonii* subsp. *berkhoffii*, was not significantly associated with either *Anaplasma* spp. or *B. burgdorferi* seroreactivity, 2 agents known to be transmitted by *Ixodes* ticks. Based on state-by-state seroreactivity rates, *B. koehlerae* exposure in dogs also appears to be more common in areas of the Rocky Mountains and Midwest where *Ixodes* ticks are uncommon, and less common in the Northeast and Middle Atlantic where *B. burgdorferi* transmission by *Ixodes scapularis* ticks is widespread. Based on this finding, studies focusing on vectors other than *Ixodes* spp. ticks should be considered for *B. koehlerae*. *Bartonella koehlerae* previously has been detected in cat fleas (*C. felis*),[6,60,61] and flea transmission should be considered for *B. koehlerae* in dogs as well.

Several limitations are inherent to retrospective seroprevalence studies. Although the motivation for submission of samples to the VBDDL is not specified on submission forms, typically most testing is performed diagnostically for sick dogs or when screening blood donors; therefore, our study sample does not represent a random sample from the general dog population in North America. The decision to submit a sample for testing may be biased by both owners and veterinarians, based on previous experience with or knowledge of *Bartonella*, as well as perception of vector-borne disease risk in certain locations or seasons. Whether testing was done to confirm a suspected clinical diagnosis, to rule out a possible underlying etiology for a clinical syndrome typically associated with *Bartonella* or another vector-borne disease, or to screen a healthy dog (eg, blood donors, military or other working dogs), is unknown. These samples, however, do not include experimental animals from research institutions, but rather diagnostic submissions only. Limited knowledge of, and access to, *Bartonella* serology testing by both dog owners and veterinarians may lead to dogs not being tested by serology for this emerging infectious disease. The population examined in our study may overestimate or underestimate the true prevalence of exposure in healthy or sick populations of dogs. Additionally, several laboratories across the country perform *Bartonella* serology testing, but we have no reason to believe that samples would be preferentially submitted to any particular laboratory for reasons related to likelihood of positive test, so this possibility likely contributes little bias to our sample.

In addition to sample submission bias, there are limitations inherent in using serology as a diagnostic test. Serology is the current gold standard for determination of exposure to *Bartonella* for both diagnostic and serosurvey purposes, but this modality has limitations.[6] Dogs experimentally infected with single species of *Bartonella* did not develop cross-reactive antibodies against other species,[62] but the extent to which serologic cross-reactivity versus co-exposure to multiple *Bartonella* species occurs in naturally infected dogs is unknown. Previous studies have shown poor associations between seroreactivity and bacteremia,[63,64] with antibody reactivity to *Bartonella* species antigens detected in ≤50% of dogs and humans in which active infection with *B. vinsonii* subsp. *berkhoffii* and *B. henselae* can be documented.[9,10] Therefore, IFA antibody testing lacks sensitivity, and, if detected, the presence of antibodies can only be used to infer prior exposure.[9,65] The seroreactivity data described our study could underestimate the true infection rate in a given population, and do not provide information on active or subclinical infection.

In summary, we report the largest North American retrospective seroepidemiologic study targeting 3 *Bartonella* species in dogs by IFA testing. The overall *B. henselae* and *B. koehlerae* seroreactivity for the dogs tested in our study was similar to that reported for other CVBD in population-wide serosurveys, whereas lower overall *B. vinsonii* subsp. *berkhoffii* seroreactivity was found. Dogs appear to be exposed to *Bartonella* spp. throughout most of North America, and seroreactivity can be detected at any time of year. Dogs exposed to other CVBD, male intact dogs, and mixed breed dogs are at higher risk for *Bartonella* exposure. Fleas and several tick species are proposed vectors for bartonellosis in dogs; our seroepidemiologic analyses suggest there may be multiple vectors or nonvectorial transmission for *Bartonella* infection in dogs, or that the primary vector may depend on local geographic, environmental, or reservoir host factors.

Footnotes

[a] Canine SNAP 4Dx or SNAP 4Dx PLUS, IDEXX Laboratories, Westbrook, ME

[b] ArcMap ArcMap v. 10.4.1, Environmental Systems Research Institute, Redlands, CA

[c] SAS v. 9.4, SAS Institute 2002–2012, SAS, Cary, NC
[d] Version 3.01, www.OpenEpi.com

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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