Members of the genus *Bartonella*, fastidious gram-negative rod-shaped hemotropic and endotheliotropic bacteria, are important emerging pathogens in dogs and humans worldwide. 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. *Bartonella* persists in erythrocytes and vascular endothelial cells, causing chronic relapsing bacteremia.}

**Abbreviations:**

| Abbreviation | Description |
|--------------|-------------|
| 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. *Bartonella vinsonii* subsp. *berkhoffii*, *B. henselae*, and *B. koehlerae* represent the most frequent species found infecting dogs in North America. All 3 of these species have been implicated as pathogenic in cases of endocarditis in dogs and have been associated with other clinical abnormalities in dogs including vasoproliferative diseases, vasculitis, myocarditis, polyarthritides, granulomatous disease (lymphadenitis, rhinitis, hepatitis), epistaxis, and neurologic diseases. 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. 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*). To date, no definitive vector has been identified for natural transmission of *Bartonella* to dogs. However, on the basis of case reports and serosurveys, surveys of arthropod vectors, and experimental data (Lappin and Breitschwerdt, unpublished data), ticks (including *Ixodes*...
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 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® test. Other antigens included in comprehensive serology panels for dogs included Ehrlichia canis, Babesia canis, Babesia gibsoni, and Rickefsiella 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 9FO-01) were passed from agar plate grown cultures into a Bartonella-permissive cell line, DHB2 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 felen-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 1:1,92. 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—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. Breeds were defined using AKC breeds; breeds that are not considered by the AKC were grouped as mixed breeds. 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.53 Boundaries were created from publicly available data from the US Census Bureau49 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 and OpenEpi.49 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.

| Region     | Tested | % Bh+ | % Bvb+ | % Bk+ | Any spp. % | % Of Total |
|------------|--------|-------|--------|-------|------------|------------|
| Canada     | 465    | 11.2  | 2.4    | 0.6   | 1.6        | 472        |
| E. N. Central | 2,051 | 42.0  | 2.0    | 0.9   | 1.6        | 2,063      |
| E. S. Central | 532   | 10.9  | 1.9    | 1.2   | 2.4        | 544        |
| Mid-Atlantic | 1,067 | 20.9  | 1.9    | 1.6   | 1.5        | 1,164      |
| Mountain   | 627    | 10.6  | 1.6    | 1.2   | 3.9        | 661        |
| New England | 356   | 14.9  | 3.9    | 1.7   | 3.3        | 367        |
| Pacific    | 521    | 15.9  | 2.9    | 1.8   | 2.4        | 619        |
| S. Atlantic | 6,421 | 116.1 | 1.8    | 1.2   | 2.0        | 6,548      |
| W. N. Central | 477  | 11.2  | 2.3    | 1.0   | 3.7        | 494        |
| W. S. Central | 2,487 | 71.2  | 2.9    | 0.6   | 3.2        | 2,506      |
| Year       |        |       |        |       |            |            |
| 2008       | 2,456  | 138.6 | 5.6    | 2.1   | —          | —          |
| 2009       | 2,460  | 50.2  | 2.0    | 0.9   | —          | —          |
| 2010       | 2,029  | 33.6  | 1.6    | 1.2   | —          | —          |
| 2011       | 1,987  | 13.0  | 0.7    | 1.0   | —          | —          |
| 2012       | 1,729  | 14.0  | 0.8    | 0.8   | —          | —          |
| 2013       | 1,920  | 29.1  | 1.5    | 1.0   | —          | —          |
| 2014       | 2,436  | 43.8  | 1.8    | 1.2   | —          | —          |
| Month      |        |       |        |       |            |            |
| December–February | 3,454 | 76.2  | 2.2    | 1.8   | 3.5        | 3,554      |
| March–May  | 3,829  | 79.2  | 2.1    | 1.2   | 2.3        | 3,940      |
| June–August | 3,994 | 83.2  | 2.1    | 1.4   | 2.0        | 4,096      |
| September–November | 3,740 | 78.2  | 2.2    | 1.2   | 2.0        | 3,861      |

Bh, B. henselae; Bvb, B. vinsonii subsp. berkhoffii; Bk, B. koehlerae; Any, seroreactive to any 1 or more Bartonella spp.
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 percent likelihood estimates with logistic regression and Table 2, given the negligible differences using the maximum likelihood estimates and Wald chi-square test. Statistical significance indicated by * at $P \leq 0.05$.

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 North Central regions (1.51, 1.56, and 1.64%, 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 (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 of the 3 * Bartonella* spp. tested.

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.

![Bartonella spp. seroreactive dogs, 2008–2014.](image)

**Fig. 1.** *Bartonella* spp. seroreactive dogs, 2008–2014. *Bh*, *B. henselae*; *Bb*, *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 species represented by *.*

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

| Region              | OR     | 95% CI        | P Value |
|---------------------|--------|---------------|---------|
| 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     | 0.0383* |

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 $P \leq 0.05$. **OR** and **95% CI** are shown for each category compared to the reference category.
(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

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**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.

**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.
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. 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, 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. 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, no Bartonella species was found...
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 VBDDDL in North Carolina. Extrapolations to under-represented 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 previously14,26,27,31,33–36 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

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

| Bartonella spp.       | OR  | 95% CI   | P Value |
|-----------------------|-----|----------|---------|
| B. henselae           | 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. koehlerae          |     |          |         |
| 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* |
| Any Bartonella spp.   |     |          |         |
| 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* |

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

Statistical significance indicated by * at P ≤ 0.05.
expected to roam, and increased contact with reservoir hosts (e.g., feral cats, wild canids such as coyotes, or their ecto-parasites).\(^{51,55,56}\) 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 sanguineus*,\(^{26,27,31,35,39,44}\) *Ixodes* spp.,\(^{28,33,36–38,40–43}\) *Dermacentor variabilis*,\(^{34,30,31,34,38}\) *Amblyomma americanum*,\(^{31,35}\) *Ancylostoma caninum*,\(^{36,37}\) or *Echinococcus granulosus*.\(^{32,35}\) However, given the likelihood of CVBD co-exposures and coinfections,\(^{52}\) 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.\(^{53,54}\) 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 *Amblyomma americanum* or *B. burgdorferi* transmission by *Ixodes* scapularis ticks. 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 in *B. koehlerae* infected dogs.\(^{55}\)

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 (e.g., 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 between 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 infections.

## 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
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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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