Evaluation of cell culture-grown *Bartonella* antigens in immunofluorescent antibody assays for the serological diagnosis of bartonellosis in dogs

Pradeep Neupane | Barbara C. Hegarty | Henry S. Marr | Ricardo G. Maggi | Adam J. Birkenheuer | Edward B. Breitschwerdt

Department of Clinical Sciences and the Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

Correspondence
Edward Breitschwerdt, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27607. Email: ed_breitschwerdt@ncsu.edu

Funding information
American Kennel Club Canine Health Foundation, Grant/Award Number: 02287

**Background:** Because of poor sensitivity and questionable specificity of immunofluorescent antibody assays (IFAs), serological diagnosis of *Bartonella* species infections in dogs remains challenging. Despite limitations, IFA testing is the historical "gold standard" for *Bartonella* serodiagnosis in animals and humans. Because most diagnostic laboratories test against only 1 or 2 *Bartonella* spp., testing against a broader panel of *Bartonella* antigens may enhance diagnostic sensitivity and specificity.

**Objective:** To evaluate the sensitivity and specificity of *Bartonella* IFA using 8 cell culture-grown *Bartonella* spp. isolates.

**Animals:** Archived serum samples from 34 *Bartonella* spp. naturally exposed, polymerase chain reaction (PCR)-positive dogs and from 26 PCR-negative and IFA-negative dogs.

**Methods:** *Bartonella* IFA sensitivity and specificity were assessed using cell culture-grown whole cell antigens derived from 3 *Bartonella henselae* (Bh) strains (Bh Houston 1, Bh San Antonio Type 2, Bh California 1), 3 *Bartonella vinsonii* subsp. *berkhoffii* genotypes (Bvb I, II, and III), *Bartonella koehlerae* (Bk), and *Bartonella quintana* (Bq).

**Results:** Only 62% of 34 *Bartonella* spp. PCR-positive dogs were seroreactive to any of the 8 *Bartonella* IFA antigens, indicating low IFA sensitivity. PCR-positive dogs were most often IFA seroreactive to Bq (n = 15), to Bvb II (n = 13), or to both (n = 9) antigens. Of the 26 previously IFA-negative/PCR-negative dogs, 4 (15%) were seroreactive using the expanded antigen panel.

**Conclusion and Clinical Importance:** Despite IFA testing of dogs against 8 different *Bartonella* isolates, IFA sensitivity remained poor, and specificity was only 85%. Development of a reliable serological assay is needed to facilitate the diagnosis of *Bartonella* infection in dogs.

**KEYWORDS**
bacteria, BAPGM, serology, vector-borne, zoonosis

---

**INTRODUCTION**

Bartonellosis is an emerging, vector-borne zoonotic disease that affects dogs throughout much of the world. *Bartonella* spp. are transmitted to mammals by arthropod vectors, including ticks, fleas, keds, lice, mites, and sand flies. Various arthropods transmit different *Bartonella* spp. among reservoir and incidental hosts, thereby...

**Abbreviations:** BAPGM, *Bartonella* alpha-proteobacteria growth medium; Bcl, *Bartonella clarridgeiae*; Bh CAL1, *Bartonella henselae* California 1; Bh H1, *Bartonella henselae* Houston 1; Bh SA2, *Bartonella henselae* San Antonio 2; Bh, *Bartonella henselae*; Bk, *Bartonella koehlerae*; Bq, *Bartonella quintana*; Br, *Bartonella rochalimae*; Bvb, *Bartonella vinsonii* subspecies *berkhoffii*; CVBD, canine vector-borne diseases; IFA, immunofluorescent antibody assay; ITS, intergenic transcribed spacer; NCSU-CVM-VBDDL, North Carolina State University, College of Veterinary Medicine, Vector Borne Diseases Diagnostic Laboratory; PBS, phosphate buffered saline; spp., species
complicating and confounding clinical, diagnostic, and epidemiological analyses. Currently, serology, as well as culture-based and polymerase chain reaction (PCR) assays, are relatively insensitive for the diagnosis of bartonellosis in dogs. Ideally, a serological assay used for bartonellosis also contributes to variation in antibody detection. Subsequently, because members of the genus Bartonella can induce long-lasting bacteremia, the stage of infection (acute, subacute, or chronic) also contributes to variation in antibody detection. Further complicating diagnoses, clinical signs, pathologic sequelae, and antibody kinetics can vary among individual animals infected with the same Bartonella strain. Because members of the genus Bartonella can induce long-lasting bacteremia, the stage of infection (acute, subacute, or chronic) also contributes to variation in antibody detection. Subjectivity associated with IFA interpretation and variability in technical variables among laboratories further contribute to differences in antibody detection or reported antibody titers. Thus, Bartonella spp. serodiagnosis is influenced by variations in bacterial, host, and laboratory variables.

Although 10 Bartonella spp. have been implicated in association with endocarditis, myocarditis, or other disease manifestations in dogs; Bartonella henselae (Bh), Bartonella koehlerae (Bk), and Bartonella vinsonii subsp. berkhoffii (Bvb) have been the most frequently documented species in North America. Historically, Bvb represented the first Bartonella spp. isolated from dogs. Therefore, initial IFA testing used Bvb as the sole antigen source for diagnostic and research purposes. Subsequently, 4 Bvb genotypes and several other Bartonella spp. were found to infect dogs, including B. clarridgeiae (Bcl), B. elizabethae, Bh, Bk, Bq, B. rochalimae (Br), B. volans, and B. wadsworthii. After natural or experimental infection with a Bartonella spp., dogs develop a species-specific IFA antibody response. However, bacteremic sick dogs frequently are seroreactive to multiple IFA antigens or alternatively they are not Bartonella spp. seroreactive despite extended illness durations. Ideally, a serological assay used for epidemiological or diagnostic purposes should detect antibodies regardless of the infecting Bartonella spp., genotype, or strain. Currently, because there are at least 38 named and Candidatus Bartonella spp., with nearly half implicated in association with infections of dogs or humans, we posed the question: Would a broader panel of Bartonella spp. antigens increase the serodiagnostic sensitivity and specificity of IFAs? We hypothesized that a comprehensive panel of Bartonella spp. isolates would increase IFA serodiagnostic sensitivity, while optimizing specificity. Therefore, the purpose of our study was to evaluate the sensitivity and specificity of 8 IFAs using archived serum samples from Bartonella spp. naturally-exposed (PCR-positive) and presumptively non-exposed (seronegative/PCR-negative) dogs.

2 MATERIALS AND METHODS

2.1 Source of sera for immunofluorescent antibody assays testing

Sixty archived sera from dogs previously tested at the North Carolina State University, College of Veterinary Medicine, Vector Borne Diseases Diagnostic Laboratory (NCSU-CVM-VBDDL) were selected for comparative IFA testing against 8 cell culture-grown Bartonella spp. antigens. Serum samples were categorized into 2 groups to assess sensitivity and specificity. All sera were submitted to the NCSU-CVM-VBDDL for diagnostic testing between 2011 and 2016. After initial processing by the NCSU-CVM-VBDDL, sera were stored at −80 °C.

2.1.1 Group I (polymerase chain reaction positive dogs)

Group I consisted of 34 stored frozen serum samples from Bartonella spp. naturally infected dogs (PCR-positive) for which the species, genotype, or strain was confirmed by DNA sequencing. We could only identify 34 Bartonella spp. seropositive dogs with adequate sera to test against all 8 antigen preparations. Veterinarians often request only serology or only PCR, thus the number of matched specimens for inclusion in our study was limited. Group I sera were used to examine the sensitivity of each of the 8 IFAs. Bartonella 16S–23S intergenic transcribed spacer (ITS) region DNA was amplified from blood (n = 31) or Bartonella alpha-Proteobacteria growth medium (BAPGM) enrichment blood culture (n = 3), after which amplicons from each dog were sequenced for the confirmation of bacterial strain and species. Dogs were infected with Bh (n = 20), Bvb (n = 6), B. vinsonii (DNA sequence incomplete to determine subsp. n = 2), Bcl (1), Br (2), Bk (2), and Bq (1). For Group I dogs, Bartonella spp. PCR-positive cases were included regardless of the tests requested by the attending clinician. Therefore, comprehensive serology and PCR testing was not available for most dogs in Group I.

2.1.2 Group II (immunofluorescent antibody assay negative and polymerase chain reaction negative dogs)

Group II consisted of 26 dogs for which diagnostic testing was negative for evidence of exposure to canine vector-borne disease (CVBD) organisms that are routinely tested for in the NCSU-CVM-VBDDL. These sera were used to assess the specificity of the 8 IFAs. Sera from all 26 dogs were PCR-negative after whole blood DNA extraction for Bartonella, Babesia, Ehrlichia, Anaplasma, Rickettsia, hemotropic Mycoplasma and Leishmania spp. In addition, all sera were IFA nonreactive (titers <1:16) to the 3 Bartonella spp. (Bh SA2, Bvb I, and Bk), Rickettsia rickettsii, Ehrlichia canis, Babesia canis, Babesia gibsoni and were not seroreactive to Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi, Ehrlichia canis, and Ehrlichia ewingii by ELISA (SNAP 4Dx PLUS ELISA, IDEXX Laboratories, Westbrook, Maine).

2.1.3 Immunofluorescent antibody assay serology assays

Bartonella spp. antibody reactivity was determined by following traditional IFA practices with fluorescein-conjugated goat anti-dog IgG (Cappel, ICN, Costa Mesa, CA), as described previously. Bartonella organisms derived from clinical isolates representative of common serotypes of Bartonella spp., specifically, Bh (strains Bh H1, Bh SA2,
and Bh CAL1), Bvb (genotypes I, II and III), Bk, and Bq each were grown in cell culture for antigen preparation. Previously frozen stocks of isolates were grown on blood agar plates. Once colonies were plentiful, bacterial cultures were passed into Bartonella-permissive cell lines, DH82 (a canine monocytoid cell line) for Bvb genotype I (NCsu 93CO-01, ATCC type strain #51672),26 Bvb genotype II (NCsu 95CO-08),27 Bh H1 (NCsu 93FO-23), Bh SA2 (NCsu 95FO-09), Bk (NCsu 09FO-01), and Bq (NCsu 11MO-01), FCR68 (a canine monocytoid cell line) for Bh CAL1 (NCsu 08HO-2424), and Vero cells (a monkey fibroblast cell line) for Bvb genotype III (NCsu 06CO-01).30 Bartonella isolates used for antigen preparation in our study had not been passaged more than 2 times. For each isolate, cellular preparations were diluted to achieve a single layer of evenly-spaced cells that were 50% to 80% infected with bacteria when layered onto 30-well Teflon-coated slides. Acetone fixation was used for pathogen inactivation, to adhere cells to the slides and to permeate cellular membranes so that secondary anti-canine fluorescein-conjugated molecules could access antigens expressed within cells.

For IFA testing, serum was diluted in phosphate buffered saline (PBS) solution containing 1% normal goat serum, 0.05% Tween-20, and 0.5% powdered nonfat dry milk to block nonspecific antigen-binding sites. Sera were screened at 1:16 to 1:64 dilutions, after which reactive sera were titered out at 2-fold dilutions to 1:8192. To avoid interpretation issues induced by nonspecific background fluorescence found at low dilutions (1:16 or 1:32), a cutoff titer of 1:64 was used to define a seroreactive titer.

2.1.4 | Positive and negative control sera

Serum from a naturally exposed dog (VB09-01611) as confirmed by prior serology testing (seroreactive to Bh H1 and Bvb I) at the NCSU-CVM-VBDDL was used as a positive IFA control. This dog’s serum was reactive to all 8 Bartonella spp. antigens with endpoint titers that varied from 1:1024 to 1:8192. The negative control serum sample also was from a diagnostic accession (VB16-06078) and was nonseroreactive and PCR-negative to the same tick-borne pathogens used to screen Group II dogs. In addition, the negative control sera was not reactive to any of the 8 Bartonella spp. antigens at 1:16, 1:32 or 1:64 dilutions.

3 | RESULTS

3.1 | Group I (polymerase chain reaction positive dogs)

Seroreactivity to each of the 8 Bartonella spp. antigens is summarized in Table 1. Sixty-two percent of Bartonella spp. PCR-positive dogs were seroreactive to at least 1 of 8 Bartonella spp. antigens, of which 41% (14/34) were seroreactive to ≥2 Bartonella antigens, whereas 38% of the PCR-positive dogs were not seroreactive to any of the 8 test antigens (Table 1). Seven PCR-positive dogs were seroreactive to only 1 Bartonella spp. antigen, of which 4, 2, and 1 dog were seroreactive to Bq, Bvb II, and Bvb III, respectively.

Among the 8 selected antigen preparations, PCR-positive dogs most often were seroreactive to Bq (44%) and Bvb II (38%; Table 1). Although Bq antigen yielded the highest sensitivity compared to other selected Bartonella antigens, there was considerable background fluorescence compared to the other IFA preparations, making technician interpretation challenging at low serum dilutions. When individual results for Bq and Bvb II seroreactivity were combined, diagnostic use of these 2 antigens would have resulted in the identification of 19/21 IFA seroreactive/PCR-positive dogs, but would not have identified seroreactivity for the remaining 13 dogs. Historically, our laboratory used only Bvb I (the first dog isolate of a Bartonella spp. and the ATCC-type strain) for serodiagnostic testing.26

The IFA sensitivities for Bvb I (35%), Bvb II (38%), and Bvb III (35%) antigens were similar (Table 1). If all the 3 Bvb genotype antigens were used for diagnostic IFA testing, 50% (17/34) of Bartonella spp. PCR-positive dogs would have been seroreactive, of which 8/17 dogs were seroreactive to all 3 (Bvb I, Bvb II, and Bvb III) genotypes. Among the 3 Bh IFA antigens, use of Bh SA2 provided the highest sensitivity (24%) followed by Bh H1 (21%) and Bh CAL1 (15%). Sensitivity of IFA would have been only 26% (9/34) if all 3 Bh antigens were used for IFA testing. Only 18% (6) of the 34 PCR-positive dogs were Bk seroreactive.

To evaluate the potential association between PCR status and seroreactivity, PCR results for Group I dogs were compared with the corresponding IFA seroreactivity. Of the 21 IFA seroreactive dogs, 5 (3 Bvb II, 1 Bcl, and 1 Br PCR-positive) were seroreactive to all 8 Bartonella spp. antigens. Of the remaining 16 dogs, 7 were seroreactive to 1, 4 to 4, 3 to 2, 1 to 6, and 1 to 3 Bartonella antigens. Eleven (55%) of 20 Bh PCR-positive dogs were not seroreactive to any of the 8 Bartonella antigens and 18/20 were not seroreactive to any of the 3 Bh antigens. Of the 8 Bvb PCR-positive dogs (4 Bvb II, 1 Bvb I, 1 Bvb III, and 2 Bvb [genotype undetermined based upon limited DNA sequence]), 7 were seroreactive to at least 1 Bartonella antigen. The Bvb I PCR-positive dog was seroreactive to Bvb I, Bvb III, and Bq. One Bvb II PCR-positive dog was not reactive to any of the 8 Bartonella antigens, whereas the remaining 3 Bvb II PCR-positive dogs were seroreactive to all 8 Bartonella antigens. One dog each, infected with Bvb III and Bvb (genotype undetermined) was IFA seroreactive to all 3 Bvb genotypes and Bq. The other Bvb (genotype undetermined) infected dog was only Bq seroreactive. Among the subset of 8 Bvb PCR-positive dogs, 6 were seroreactive to at least 2 of the 3 Bvb antigens in the panel, whereas 2/8 were not seroreactive to any of the 3 Bvb antigens.

3.2 | Group II (immunofluorescent antibody assay negative/polymerase chain reaction negative dogs)

Of the 26 PCR-negative nonseroreactive dogs, 22 dogs (85%) were not seroreactive to any of the 8 Bartonella IFA antigens (Table 1). One dog was seroreactive to Bvb II, Bvb III, Bh H1, Bh SA2, and Bh CAL1, and 1 dog each was seroreactive to either Bvb II, Bvb III, or Bq antigens. No Group II dog was seroreactive to Bvb I or Bk antigens.

4 | DISCUSSION

In our study, sera from Bartonella PCR-positive dogs varied from nonseroreactive to strongly seroreactive to all 8 IFA antigen preparations.
### Table 1: Comparison of dog sera IFA seroreactivity with PCR assay

| Groups | PCR results (N = PCR positive) | Number of Bartonella spp. seroreactors | Number of dogs nonseroreactive to all 8 antigens | Dogs seroreactive to any Bh Ag | Dogs seroreactive to any Bvb Ag | Total dogs seroreactive to any Bartonella spp. |
|--------|-------------------------------|----------------------------------------|-----------------------------------------------|-------------------------------|---------------------------------|-----------------------------------------------|
| Group I |                                | Bh H1 | Bh SA2 | Bh CAL1 | Bvb I | Bvb II | Bvb III | Bk | Bq | | |
| Bh (20) |                               | 2 | 1 | 0 | 2 | 4 | 3 | 0 | 3 | 11 | |
| Bvb I (1) |                             | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | |
| Bvb II (4) |                             | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | |
| Bvb III (1) |                             | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | |
| Bvb (2)* |                             | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 2 | 0 | |
| Bk (2) |                               | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 2 | 0 | |
| Bq (1) |                               | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| BcI (1) |                               | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | |
| Br (2) |                               | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 0 | |
| Total |                               | 7 | 8 | 5 | 12 | 13 | 12 | 6 | 15 | 13 | |

**Group II** PCR-negative

|                                | 1 | 1 | 1 | None | 2 | 1 | None | 1* | 22 | 1 | 3 | 4 |
|--------------------------------|---|---|---|------|---|---|------|----|----|---|---|---|
| Number                        | 4%| 4%| 4%|      | 4%| 4%|      | 4% |    | 4%| 4%| 15%|

Comparison of IFA seroreactivity results among dogs infected with various *Bartonella* species, subspecies or strains. Serology results for each of the eight IFA antigens were compared with *Bartonella* spp. PCR/DNA sequencing status. Results are reported as the number of dogs that were seroreactive to each antigen (antibody titers ≥1:64) or were not seroreactive to any of the 8 antigens (IFA antibody titers ≤1:32). **Group I** (*Bartonella* spp. PCR+) and **Group II** (*Bartonella* spp. IFA negative and PCR negative) dogs are reported separately. Results for each group are reported as the number and percentage of dogs that were seroreactive (antibody titers ≥1:64). * represents number of dogs that were seroreactive only to that Bartonella antigen by IFA testing.

*ITS DNA sequence inadequate to genotype.*

**Abbreviations:** Ag: Antigen; Bd: *Bartonella clarridgeiae*; Bh CAL1: *Bartonella henselae* California 1; Bh H1: *Bartonella henselae* Houston 1; Bh SA2: *Bartonella henselae* San Antonio 2; Bh: *Bartonella henselae*; Bk: *Bartonella koehlerae*; Br: *Bartonella rochalimae*; Bvb: *Bartonella vinsonii* subspecies berkoffii; Bvb I: *Bartonella vinsonii* subspecies berkoffii genotype I; Bvb II: *Bartonella vinsonii* subspecies berkoffii genotype II; Bvb III: *Bartonella vinsonii* subspecies berkoffii genotype III; Bq: *Bartonella quintana*, spp: species; IFA: immunofluorescent antibody assay; ITS: intergenic transcribed spacer; PCR: polymerase chain reaction.
Despite testing against 8 Bartonella spp. or strains, only 62% of Bartonella spp. PCR-positive dogs were seroreactive to any test antigen. Based on our results, increasing the number of Bartonella spp. IFA antigens is not a practical approach to increase IFA sensitivity for the serodiagnosis of bartonellosis. Increasing the number of antigens would substantially increase diagnostic testing costs associated with the time and materials required for antigen growth in cell culture followed by IFA slide preparation, increased IFA conjugate requirements, and substantially increased technician time for interpretation of each antigen preparation. Of the 8 IFA assays examined, seroreactivity most often was documented to Bq (44%), followed by Bvb II (38%), Bvb I (35%), Bvb III (35%), and Bh SA2 (24%). In contrast to poor antigenic sensitivity, IFA specificity to the panel of 8 antigens was at least 85% in 26 Bartonella spp. PCR-negative dogs. Similar to the findings reported in previous studies,4,8 our study documented a lack of agreement between infection (PCR or BAPGM culture/PCR) and IFA seroreactivity (infection or exposure).

Among the subset of PCR-positive/IFA seroreactive dogs, 90% (19/21) would have been identified if tested using only the Bvb II and Bq IFA assays, but 13 Group I dogs were not Bvb II or Bq seroreactive, further illustrating IFA sensitivity limitations. Clinically, Bq and all 4 Bvb genotypes have been isolated or detected in dogs or humans with endocarditis, myocarditis, and neurological diseases.26–32 Although Bvb I was the first Bartonella spp. isolated from a dog, Bvb II was subsequently found to be a more frequent infection in dogs and humans tested in our diagnostic (dogs) or research laboratory (humans).29,32 Bq has been isolated from 2 dogs with endocarditis,33 from Ctenocephalides felis, the common cat and dog flea,34 and from a woman and subsequently from the feral cat that bit her.35 Collectively, these and other observations suggest that the epidemiology of Bq in dogs, a historically important Bartonella spp. that caused Trench Fever in WWI, should be reassessed, particularly in light of the possibility that cat fleas and head lice, as well as the human body louse may transmit this organism.36,37

Despite testing dogs against 3 different Bh strains, only 10% of the 20 Bh PCR-positive dogs were Bh IFA seroreactive, which is even less than noted in a previous study9 in which only 25% of Bh-infected dogs were Bh IFA seroreactive to a single Bh strain. In contrast to the Bh IFA results, 9 of 20 Bh bacteremic dogs were seroreactive (IFA titers ≥1:64) to at least 1 of the 3 Bvb genotypes, Bq antigens, or both. Whether these disparate IFA results in Bh PCR-positive dogs reflect serological cross-reactivity or prior exposure to other Bartonella spp. is unknown. In contrast to the overall lack of seroreactivity among Bh-infected dogs, most dogs that were PCR-positive for Br, Bcl, and Bvb II were seroreactive to all 8 Bartonella antigens, including reactivity (Bh titer ≥1:64) to all 3 Bh strains. Collectively, our results indicate that seroreactivity patterns in PCR-positive dogs are highly variable and often do not correlate with the organism that was PCR amplified and sequenced from their blood.

Previously, we documented a lack of cross-reactivity to Bartonella spp. antigens when testing sera from dogs experimentally infected with Rickettsia rickettsii or Ehrlichia canis, 2 organisms that are alpha proteobacteria phylogenetically closely related to Bartonella spp. in the evolutionary microbial tree of life.12,15,38 Also, cross-reactivity among Bh, Bvb, and Bk antigens did not occur in 2 dogs infected with each of these 3 Bartonella spp.28 Canine vector-borne disease (CVBD) serology results (15 451 diagnostic submissions) generated between January 1, 2008 and December 31, 2014 at the NCSU-CVM-VBDDL recently were retrospectively reviewed.39 Bh (2.13%), Bk (2.39%), and Bvb I (1.42%, P < 0.0001) seroreactivities among dogs tested because of suspicion of a vector-borne disease were low, further supporting the specificity of Bartonella spp. IFA testing.

In an effort to further examine IFA specificity, we assessed sera from 26 dogs that were PCR-negative/IFA-negative to our panel of vector-borne pathogens and IFA nonseroreactive to the 3 Bartonella spp. (Bh SA2, Bvb I, and Bk) used diagnostically in the NCSU-CVM-VBDDL. When tested against the 8 Bartonella antigens, 85% (22/26) were not seroreactive to any antigen and no dog was seroreactive to Bvb I or Bk IFA antigens routinely used for serodiagnostic testing of dogs in our laboratory. Collectively, our research to date supports low sensitivity, but relatively good specificity for the historical panel of Bartonella spp. antigens used in the NCSU-CVM-VBDDL. Interestingly, 3 Group II dogs were only Bq, Bvb II, or Bvb III seroreactive, respectively. If Bq, Bvb II, or Bvb III seroreactivity reflects exposure solely to Bq, Bvb II, or Bvb III, rather than nonspecific IFA immunofluorescence, our historical antigen panel has most likely underestimated Bartonella seroprevalence in dogs.15,39 The remaining PCR-negative/IFA-negative dog was seroreactive to Bvb II and III, Bh H1, and Bh SA2. This dog was previously reported IFA-negative using the NCSU-CVM-VBDDL antigen preparations. This finding illustrates that IFA antibody titers should be interpreted with caution because of variability among antigen preparations (e.g., the species or strain used for testing), immunoglobulin conjugates, and the diagnostic technician’s interpretation of specific versus nonspecific immunofluorescence.

Although serology, culture, and PCR remain the mainstays for diagnosing bartonellosis in dogs, clinicians and diagnosticians should recognize that each of these assays lacks sensitivity, specificity, or both.5,40,41 These limitations adversely influence seroepidemiological conclusions; potentially result in the transfusion of Bartonella spp. bacteremic donor blood into sick dogs; impede attainment of an accurate microbiological diagnosis upon which to base-directed therapy; and, may adversely influence patient outcomes. Although an effective antibiotic regimen for the treatment of bartonellosis in dogs has not been established, treatment failures have been associated with both short-term and long-term antibiotic administration.27,42 Thus, an accurate diagnosis of bartonellosis in dogs is critical to facilitate directed antimicrobial treatment, and to avoid the unnecessary use of antibiotics that may contribute to antimicrobial resistance.

In conclusion, we report comparative data for 8 cell culture-grown Bartonella isolates used in IFAs for serodiagnosis of bartonellosis. With the rapid expansion of characterized Bartonella spp., many of which appear to be pathogenic for dogs, a serological assay that confirms exposure to any Bartonella spp. would be optimal. Currently, there are no highly sensitive serological assays to determine if a dog has been exposed to or is infected with a Bartonella spp. Determining which Bartonella spp., genotype or strain a dog has been exposed to may have important epidemiological or zoonotic implications. Based on existing knowledge, however, these phylogenetic classifications currently do not influence antibiotic selection, treatment duration, or prognosis. Increasing the number of Bartonella spp., genotypes or
strains used in IFA panels is technically time consuming, increases the cost of diagnostic testing and, based upon the results of our study, does not substantially enhance overall diagnostic sensitivity. Our findings will be used in future research efforts aimed at improving the serodiagnosis of Bartonella spp. infections in dogs.

ACKNOWLEDGMENTS

The authors are grateful to NCSU-CVM-VBDDL for providing characterized canine serum samples, without which our study would not have been possible. The authors thank Julie Bradley and Kaye Gore for technical assistance. We also thank Nandhu Balakrishnan and Erin Lashnits for helpful comments and discussion of the study results. Our study was performed at North Carolina State University, College of Veterinary Medicine, Vector Borne Diseases Diagnostic Laboratory (NCSU-CVM-VBDDL). Our research was funded by the American Kennel Club Canine Health Foundation Grant # 02287: Enhanced Serological Testing Modalities for the Diagnosis of Bartonellosis in Dogs.

CONFLICT OF INTEREST DECLARATION

Authors declare no 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

Samples were obtained from the diagnostic sample back at the Vector Borne Disease Diagnostic Laboratory at North Carolina State University, College of Veterinary Medicine so no IACUC was required.

ORCID

Barbara C Hegarty https://orcid.org/0000-0002-5179-3720
Ricardo G Maggi https://orcid.org/0000-0001-7428-0138
Adam J Birkenheuer https://orcid.org/0000-0002-2617-2252
Edward B Breitschwerdt https://orcid.org/0000-0002-3506-0279

REFERENCES

1. Breitschwerdt EB, Maggi RG, Chomel BB, Lappin MR. Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. J Vet Emerg Crit Care (San Antonio). 2010;20:8-30.
2. Breitschwerdt EB, Kordick DL. Bartonella infection in animals: carrier-ship, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13:428-438.
3. Breitschwerdt EB. Bartonellosis, one health and all creatures great and small. Adv Vet Dermatol. 2017;28(1):96-e21.
4. Balakrishnan N, Musulin S, Varanat M, Bradley JM, Breitschwerdt EB. Serological and molecular prevalence of selected canine vector borne pathogens in blood donor candidates, clinically healthy volunteers, and stray dogs in North Carolina. Parasit Vectors. 2014;7(1):116.
5. Maggi RG, Birkenheuer AJ, Hegarty BC, Bradley JM, Levy MG, Breitschwerdt EB. Comparison of serological and molecular panels for diagnosis of vector-borne diseases in dogs. Parasit Vectors. 2014;7(1):127.
6. Beerlage C, Varanat M, Linder K, et al. Bartonella vinsonii subsp. berkhoffii and Bartonella henselae as potential causes of proliferative vascular diseases in animals. Med Microbiol Immunol. 2012;201(3):319-326.
7. Okaro U, Addisu A, Casanas B, Anderson B. Bartonella species, an emerging cause of blood-culture-negative endocarditis. Clin Microbiol Rev. 2017;30(3):709-746.
8. Pérez C, Maggi RG, Díez PP, Breitschwerdt EB. Molecular and serological diagnosis of Bartonella infection in 61 dogs from the United States. J Vet Intern Med. 2011;25(4):805-810.
9. Henn JB, Liu CH, Kasten RW, et al. Seroprevalence of antibodies against Bartonella species and evaluation of risk factors and clinical signs associated with seropositivity in dogs. Am J Vet Res. 2005;66(4):688-694.
10. Duncan AW, Maggi RG, Breitschwerdt EB. A combined approach for the enhanced detection and isolation of Bartonella species in dog blood samples: pre-enrichment culture followed by PCR and subculture onto agar plates. J Microbiol Methods. 2007;69:273-281.
11. Díez PP, Morton BA, Tngrian M, et al. Infection of domestic dogs in Peru by zoonotic Bartonella species: a cross-sectional prevalence study of 219 asymptomatic dogs. PLoS Negl Trop Dis. 2013;7(9):e2393.
12. Hegarty BC, Bradley JM, Lappin MR, Balakrishnan N, Mascarelli PE, Breitschwerdt EB. Analysis of seroreactivity against cell culture-derived Bartonella spp. antigens in dogs. J Vet Intern Med. 2013;28(1):38-41.
13. Vermeulen MJ, Verbakel H, Notermans DW, Reimerink JH, Peeters MF. Evaluation of sensitivity, specificity and cross-reactivity in Bartonella henselae serology. J Med Microbiol. 2010;59(6):743-745.
14. Sander A, Berner R, Ruess M. Serodiagnosis of cat scratch disease: response to Bartonella henselae in children and a review of diagnostic methods. Eur J Clin Microbiol Infect Dis. 2001;20(6):392-401.
15. Solano-Gallego L, Bradley J, Hegarty B, Sigmund B, Breitschwerdt E. Bartonella henselae IgG antibodies are prevalent in dogs from southeastern USA, Vet Res. 2004;35(5):585-595.
16. Vermeulen MJ, Herremans M, Verbakel H, et al. Serological testing for Bartonella henselae infections in The Netherlands: clinical evaluation of immunofluorescence assay and ELISA. Clin Microbiol Infect. 2007;13(6):627-634.
17. Agan BK, Dolan M. Laboratory diagnosis of Bartonella infections. Clin Lab Med. 2002;22(4):937-962.
18. Chomel BB, Abbott RC, Kasten RW, et al. Bartonella henselae prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. J Clin Microbiol. 1995;33(9):2445-2450.
19. Berghoff J, Viezens J, Guitacci L, Fabbi M, Arvand M. Bartonella henselae exists as a mosaic of different genetic variants in the infected host. Microbiology. 2007;153(7):2045-2051.
20. Breitschwerdt EB. Bartonellosis: one health perspectives for an emerging infectious disease. ILAR J. 2014;55(1):46-58.
21. Regier Y, O’Rourke F, Kempf VAJ. Bartonella spp. - a chance to establish one health concepts in veterinary and human medicine. Parasit Vectors. 2016;9(1):261.
22. Guitacci L, Slater L, Wu CC, et al. Experimental infection of young specific pathogen-free cats with Bartonella henselae. J Infect Dis. 1997;176:206-216.
23. Yamamoto K, Chomel BB, Kasten RW, Hew CM, Weber DK, Lee WD. Experimental infection of specific pathogen free (SPF) cats with two different strains of Bartonella henselae type I: a comparative study. Vet Res. 2002;33(6):669-684.
24. Kordick DL, Brown TT, Shin K, Breitschwerdt EB. Clinical and pathologic evaluation of chronic Bartonella henselae or Bartonella claridgeiae infection in cats. J Clin Microbiol. 1999;37(5):1536-1547.
25. Minnick MF, Battisti JM. Pestilence, persistence and pathogenicity: infection strategies of Bartonella. Future Microbiol. 2009;4(6):743-758.
26. Breitschwerdt EB, Kordick DL, Malarkey DE, Keene B, Hadfield TL, Wilson K. Endocarditis in a dog due to infection with a novel Bartonella subspecies. J Clin Microbiol. 1995;33(1):154-160.
27. Chomel BB, Kasten RW. Bartonellosis, an increasingly recognized zoonosis. J Appl Microbiol. 2010;109(3):743-750.

28. Balakrishnan N, Cherry NA, Linder KE, et al. Experimental infection of dogs with Bartonella henselae and Bartonella vinsonii subsp. berkhoffii. Vet Immunol Immunopathol. 2013;156:153-158.

29. Kordick DL, Breitschwerdt EB. Persistent infection of pets within a household with three Bartonella species. Emerg Infect Dis. 1998;4:325-328.

30. Cadenas MB, Bradley J, Maggi RG, Takara M, Hegarty BC, Breitschwerdt EB. Molecular characterization of Bartonella vinsonii subsp. berkhoffii genotype III. J Clin Microbiol. 2008;46:1858-1860.

31. Breitschwerdt EB, Maggi RG, Nicholson WL, Hegarty BC, Woods CW. Bartonella species in blood of immunocompetent persons with animal and arthropod contact. Emerg Infect Dis. 2007;13:938-941.

32. Breitschwerdt EB, Maggi RG, Nicholson WL, Cherry NA, Woods CW. Bartonella spp. bacteremia in patients with neurological and neuro-cognitive dysfunction. J Clin Microbiol. 2008;46(9):2856-2861.

33. Kelly P, Rolain JM, Maggi R, et al. Bartonella quintana endocarditis in dogs. Emerg Infect Dis. 2006;12:1869-1872.

34. Rolain JM, Franc M, Davoust B, et al. Molecular detection of Bartonella quintana, B. koehlerae, B. henselae, B. claridgeiae, Rickettsia felis, and Wolbachia piipientis in cat fleas, France. Emerg Infect Dis. 2003;9(3):339.

35. Breitschwerdt EB, Maggi RG, Sigmon B, Nicholson WL. Isolation of Bartonella quintana from a woman and a cat following putative bite transmission. J Clin Microbiol. 2006;45(1):270-272.

36. Fournier PE, Ndihokubwayo JB, Guiran J, Kelly PJ, Raoult D. Human pathogens in body and head lice. Emerg Infect Dis. 2002;8(12):1515-1518.

37. Diatta G, Mediannikov O, Bassene H, et al. Prevalence of Bartonella quintana in patients with fever and head lice from rural areas of Sine-Saloum, Senegal. Am J Trop Med Hyg. 2014;91(2):291-293.

38. Pappalardo BL, Correa MT, York CC, Peat CY, Breitschwerdt EB. Epidemiologic evaluation of the risk factors associated with exposure and seroreactivity to Bartonella vinsonii in dogs. Am J Vet Res. 1997;58(5):467-471.

39. Lashnits E, Correa M, Hegarty BC, Birkenheuer A, Breitschwerdt EB. Bartonella seroepidemiology in dogs from North Carolina, 2008-2014. J Vet Intern Med. 2017;32(1):222-231.

40. Hansmann Y, Demartino S, Piemont Y, et al. Diagnosis of cat scratch disease with detection of Bartonella henselae by PCR: a study of patients with lymph node enlargement. J Clin Microbiol. 2005;43(8):3800-3806.

41. Chondrogiannis K, Vezakis A, Derpapas M, Melemeni A, Fragulidis G. Seronegative cat-scratch disease diagnosed by PCR detection of Bartonella henselae DNA in lymph node samples. Braz J Infect Dis. 2012;16(1):96-99.

42. Breitschwerdt EB, Maggi RG. A confusing case of canine vector-borne disease: clinical signs and progression in a dog co-infected with Ehrlichia canis and Bartonella vinsonii subsp. berkhoffii. Parasit Vectors. 2009;2(Suppl 1):S3.