J Vet Intern Med 2011;25:805–810

Molecular and Serological Diagnosis of Bartonella Infection in 61 Dogs from the United States

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Background: Molecular diagnosis of canine bartonellosis can be extremely challenging and often requires the use of an enrichment culture approach followed by PCR amplification of bacterial DNA.

Hypotheses: (1) The use of enrichment culture with PCR will increase molecular detection of bacteremia and will expand the diversity of *Bartonella* species detected. (2) Serological testing for *Bartonella henselae* and *Bartonella vinsonii* subsp. *berkhoffii* does not correlate with documentation of bacteremia.

Animals: Between 2003 and 2009, 924 samples from 663 dogs were submitted to the North Carolina State University, College of Veterinary Medicine, Vector Borne Diseases Diagnostic Laboratory for diagnostic testing with the *Bartonella* α-*Proteobacteria* growth medium (BAPGM) platform. Test results and medical records of those dogs were retrospectively reviewed.

Methods: PCR amplification of *Bartonella* sp. DNA after extraction from patient samples was compared with PCR after BAPGM enrichment culture. Indirect immunofluorescent antibody assays, used to detect *B. henselae* and *B. vinsonii* subsp. *berkhoffii* antibodies, were compared with PCR.

Results: Sixty-one of 663 dogs were culture positive or had *Bartonella* DNA detected by PCR, including *B. henselae* (30/61), *B. vinsonii* subsp. *berkhoffii* (17/61), *Bartonella koehlerae* (7/61), *Bartonella volans-like* (2/61), and *Bartonella bovis* (2/61). Coinfection with more than 1 *Bartonella* sp. was documented in 9/61 dogs. BAPGM culture was required for PCR detection in 32/61 cases. Only 7/19 and 4/10 infected dogs tested by IFA were *B. henselae* and *B. vinsonii* subsp. *berkhoffii* seroreactive, respectively.

Conclusions and Clinical Importance: Dogs were most often infected with *B. henselae* or *B. vinsonii* subsp. *berkhoffii* based on PCR and enrichment culture, coinfection was documented, and various *Bartonella* species were identified. Most infected dogs did not have detectable *Bartonella* antibodies.

Key words: Bacteria; BAPGM; Bartonella; Blood culture.

Members of the genus *Bartonella* are fastidious Gram-negative hemotropic bacteria that are transmitted by several arthropod vectors, by blood transfusion, or via animal scratches or bites.[1,2] A unique peculiarity of this genus is the ability to cause long-lasting intravascular infection in conjunction with a relapsing pattern of bacteremia in humans, cats, and potentially other mammals.[1–3] The genus *Bartonella* contains a rapidly increasing number of recognized species, many of which are considered emerging animal and human pathogens. Because of the fastidious nature and intracellular tropism of these bacteria for erythrocytes, endothelial cells, and potentially mononuclear phagocytes, diagnostic confirmation of *Bartonella* infection has proven to be extremely challenging. Conventional diagnostic tests, such as bacterial isolation on agar plates, ELISA, and immunofluorescent antibody assays (IFA) for detection of *Bartonella* spp. antibodies, and PCR amplification of *Bartonella* DNA after direct extraction from patient samples have considerable limitations, making these tests relatively insensitive.[4] For example, in 2 small case series, approximately 50% of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* infected dogs did not have detectable IFA antibodies to the infecting *Bartonella* sp.[5,6] One objective of this retrospective study was to compare the results of *Bartonella* IFA seroreactivity to results of testing with the BAPGM enrichment platform.

Abbreviations:

BAPGM	*Bartonella* α-*Proteobacteria* growth medium
CSF	cerebrospinal fluid
EDTA	ethylene diaminetetraacetic acid
IFA	immunofluorescent antibody assays
NCSU-CVM-VBDDL	North Carolina State University, College of Veterinary Medicine, Vector Borne Diseases Diagnostic Laboratory

In 2004, we described a unique diagnostic platform that includes *Bartonella* PCR after direct extraction of DNA from the patient sample, PCR after enrichment culture in an optimized insect cell culture-based growth medium (*Bartonella* α-*Proteobacteria* growth medium [BAPGM]), and PCR if visible growth occurs after subculture of the BAPGM-enriched sample onto a blood agar plate, which is incubated for 4 weeks.[4] This diagnostic platform was developed because conventional microbiological approaches had in most instances failed to isolate *Bartonella* spp. In addition, unless dogs were therapeutically immunocompromised, PCR after direct extraction of DNA from blood samples generally failed to detect *Bartonella* spp. DNA. As an insect cell culture-based liquid growth medium, BAPGM supports the

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Submitted September 13, 2010; Revised March 16, 2011; Accepted March 22, 2011.

10.1111/j.1939-1676.2011.0736.x

growth of at least 7 *Bartonella* species.[4] More recently, the utility of insect cell culture-based media for the growth of *Bartonella* spp. has been supported by additional experimental evidence generated by researchers in Germany.[7] Utilization of the BAPGM platform has increased the diagnostic sensitivity of *Bartonella* spp. detection in blood, aseptically obtained tissue biopsy samples, and various diagnostic fluid specimens including cerebrospinal (CSF), thoracic, abdominal, pericardial, aqueous, seroma, and joint fluids.[6,8–13] Furthermore, during the development and validation of the BAPGM platform, bacteremia with 4 *Bartonella* spp. (*B. henselae*, *B. vinsonii* subsp. *berkhoffii*, *Bartonella quintana*, and *Bartonella bovis*) was documented in dogs.[8] Subsequently, BAPGM was used to achieve successful isolation of *Candidatus* Bartonella tamiae from febrile human patients in Thailand.[9]

The objectives of this study were to (1) describe the spectrum of *Bartonella* species identified in diagnostic samples from sick dogs; (2) compare the results of PCR after extraction from the patient sample to PCR results obtained after BAPGM enrichment culture with blood and other diagnostic samples; and (3) compare the results of *Bartonella* IFA seroreactivity to results of testing with the BAPGM enrichment platform in diagnostic samples from sick dogs submitted to the North Carolina State University, College of Veterinary Medicine, Vector Borne Diseases Diagnostic Laboratory (NCSU-CVM-VBDDL).

Materials and Methods

Study Population

Data from laboratory records for 924 diagnostic samples (blood, urine, CSF, thoracic, abdominal, pericardial, seroma, and joint fluids, and aseptically obtained tissue biopsy samples) from 663 sick dogs that had been submitted to the NCSU-CVM-VBDDL were tested with the BAPGM platform. In addition, serology for *B. henselae* and *B. vinsonii* subsp. *berkhoffii* was performed, when serum samples were submitted concurrently. From the VBDDL database, all dogs in which infection with a *Bartonella* sp. was confirmed by PCR amplification and DNA sequencing before or after enrichment culture in BAPGM were included in this study. The cumulative study population included 9 dogs from previously published case reports.[6,10,11]

Review of Medical Records

Medical records from each dog were reviewed. Data collected included duration of illness as well as concurrent administration of antibiotics or corticosteroids at the time of diagnosis of bartonellosis. For those samples submitted from other institutions, complete medical records were requested by e-mail, fax, or telephone, and reviewed retrospectively.

BAPGM Culture

The BAPGM diagnostic platform incorporates PCR amplification of *Bartonella* spp. DNA in 3 sample sources (blood, enrichment culture, and subculture isolate), as described previously.[8] A portion of each diagnostic sample (ranging from 200 μL to 2 mL of aseptically obtained ethylene diaminetetraacetic acid [EDTA]-anticoagulated blood or body fluid) was inoculated into liquid medium (BAPGM) at a ratio of 1 : 10 and incubated as described previously.[8] For processing of surgical biopsy tissues, each specimen was placed into a sterile Petri dish and sliced into small 1–2 mm pieces with a sterile scalpel blade. Slicing of tissue specimens was performed to maximize surface area contact with the BAPGM culture medium. Typically, between 0.5 and 1 g of tissue was used for cultures (with up to 5 mL of BAPGM). Culture conditions were identical to those used for enrichment blood culture. After a 7–10-day incubation period, a 1 mL aliquot of liquid culture medium was subinoculated onto 10% blood agar plates (TSA with 10% sheep blood) and incubated for 4 weeks. Multiple bacterial colonies were collectively swabbed from the surface of the blood agar plate, resuspended in sucrose-phosphate glutamate buffer, and stored at −80°C until processed for DNA extraction.

DNA Extraction, Quality Control, and Bartonella PCR Assay

Total nucleic acids were extracted from diagnostic specimens, BAPGM liquid culture samples, and blood agar plate colonies with the QIAamp DNA minikit.[a] All samples were eluted in nuclease-free water and stored at −20°C until use. An uninoculated BAPGM culture flask was processed simultaneously and in an identical manner with each batch of patient samples tested. PCR screening for *Bartonella* DNA was performed targeting the intergenic spacer (between 16S sRNA and 23S rRNA region) using primers sets 325s-1100as and 438s-1100as (BsppITS438s: 5′-GGTTTTCCGGTTTATCCCGGAGGGC-3′ and BsppITS1100as: 5′-GAACCGACGACCCCCTGCTTGCAAAGCA-3′) as described previously.[4,14] Primer set 438s-1100as, tested and validated at the Intracellular Pathogens Research Laboratory, NCSU, showed a detection sensitivity of 2.5 genome copies on 98% of *B. henselae* positive controls.

DNA Sequencing and Analysis

All PCR products were sequenced directly or after cloning as described previously.[15] Bacterial species and strain were defined by comparing similarities with other sequenced bacteria deposited in the GenBank database by the Basic Local Alignment Search Tool.[b]

Serological Analyses

Serology was performed using modifications of a previously described indirect fluorescent antibody test.[15] *B. vinsonii* subsp. *berkhoffii* and *B. henselae* antibodies were determined after traditional IFA practices with fluorescein conjugated goat anti-dog IgG. *B. vinsonii* subsp. *berkhoffii* genotype I (isolate 93-CO-1 from the NCSU-IPRL, ATCC #51672) and *B. henselae* (strain Houston-1, ATCC #49882) were passed from agar grown cultures of each organism into DH82 (a continuous canine histiocytic cell line) cultures. Heavily infected cell cultures were spotted onto 30-well Teflon coated slides,[c] air dried, acetone fixed, and stored frozen. Serum samples were diluted in phosphate-buffered saline solution containing normal goat serum, Tween-20, and powdered nonfat dry milk to block nonspecific antigen binding sites. Patient sera were screened at dilutions of 1 : 16–1 : 64. The cut-off titers were 1 : 64. All sera that remained reactive at a titer of 1 : 64 were further tested with 2-fold dilutions out to a final dilution of 1 : 8,192.

Statistical Analysis

Data from medical records were tabulated and analyzed by statistical software.[d] Results from PCR amplifications of *Bartonella* DNA from BAPGM liquid culture and plate isolates were combined as a single group (BAPGM group) to be compared with PCR results from original samples. PCR results from body fluids such as pleural effusion, pericardial effusion, synovial liquid, CSF, and urine were combined in a single group to be compared against blood and tissue samples. Serology results were defined as positive and negative based on the cut-off titers. Illness duration was classi-

fied into 2 categories: ≤1 month (acute) and >1 month of duration (chronic). The following variables were considered for association analysis: *Bartonella* spp. infection status, *Bartonella* spp. exposure status, specimen type (blood, tissue, body fluids), illness duration, antibiotic therapy, and corticosteroid therapy. Despite testing multiple samples from some subjects, each dog was considered as a unique event for analysis. Univariate associations initially were evaluated by χ^2 or Fisher's exact test at a significance level of 0.2. When potential associations were detected, a multivariable logistic regression was performed, with significance level of .05. Tests of agreement between matched data (PCR from original samples compared with PCR after enrichment culture, PCR results compared with serology results) were performed by the nonparametric test of McNemar with the continuity correction, with a null hypothesis of agreement between both tests, and a significance level of .05. Level of agreement between these diagnostic techniques was determined by Cohen's κ coefficient and 95% confidence intervals (CI).

Results

Of the 663 dogs tested with the BAPGM platform, 61 dogs (9.2%) were *Bartonella* PCR positive at 1 or more testing steps (Table 1). Twenty-eight dogs were examined at NCSU-CVM-Veterinary Teaching Hospital and 33 at other veterinary teaching hospitals or private veterinary clinics. A total of 924 diagnostic samples were submitted from North Carolina, South Carolina, New York, Virginia, Minnesota, Tennessee, Texas, Connecticut, Mississippi, Pennsylvania, and New Hampshire. For 44/61 (72.1%) dogs, the duration of illness was ≤1 month, for 4/61 (6.6%) dogs between 3 and 6 months and for 13/61 (21.3%) dogs >6 months. Twenty-seven of 61 (44.3%) dogs were receiving at least 1 antibiotic at the time of *Bartonella* testing, 19 of which were being treated with multiple antibiotics. Antimicrobials included tetracyclines (4/27, 14.8% of dogs), macrolides (20/27, 74%), rifamycins (6/27, 22.2%), fluoroquinolones (8/27, 29.6%), and cephalosporins (4/27, 14.8%). Four of 61 dogs (6.6%) dogs were treated with corticosteroids. *Bartonella* sp. DNA was amplified and sequenced directly from extracted blood, tissue, or body fluids, from BAPGM enrichment cultures, or from agar plate isolates for 29 (47.5%), 29 (47.5%), and 16 (26.2%) specimens, respectively. *Bartonella* spp. DNA was not amplified from any negative control sample at any time during the study. Forty dogs (66.6%) were PCR-positive after enrichment culture, of which only 8/61 dogs (13.1%) were also PCR-positive after extraction of sample DNA. Thirty-two dogs (52.5%) had a positive diagnostic sample for *Bartonella* DNA only after the BAPGM enrichment steps. The BAPGM platform diagnosed 52.4% more dogs infected with *Bartonella* spp. than PCR from the original sample, but this difference was not significant ($P = .17$, McNemar test). The Cohen κ coefficient of agreement between PCR from original samples and BAPGM platform was 0.19 (95% CI of 0.05–0.32), which is considered a slight agreement. Of the 61 infected dogs, *B. henselae* was amplified and sequenced from 30/61 (49.2%), *B. vinsonii* subsp. *berkhoffii* from 17/61 (27.9%), *B. koehlerae* from 2/61(3.2%), *Bartonella volans-like* from 2/61 (3.2%), and *B. bovis* from 1/61 (1.6%). The remaining 9 (14.8%) dogs were coinfected with >1 *Bartonella* species (Table 1). Infection with ≥1 *Bartonella* spp. was confirmed by PCR amplification and DNA sequencing in 61 blood and 6 serum samples. *Bartonella* sp. infection was detected in 18 tissue samples, and 9 body fluid or effusion samples (thoracic, pleural, peritoneal, seroma, joint fluid, CSF, and urine). Of 18 PCR-positive tissue samples (liver, pancreas, lymph node, bone marrow, lung, and heart), 12 (66.6%) contained *B. henselae*, 2 (11.1%) contained *B. vinsonii* subsp. *berkhoffii*, 2 (11.1%) were coinfected with *B. henselae*, and *B. vinsonii* subsp. *berkhoffii*, and 2 (11.1%) dogs were infected with *B. koehlerae*. Of the 9 PCR positive effusion or fluid samples, 4 (44.4%) contained *B. henselae*, 3 (33.3%) contained *B. vinsonii* subsp. *berkhoffii*, and 2 (22.2%) contained *B. henselae and B. vinsonii* subsp. *berkhoffii*. When compared with other *Bartonella* species or coinfections with multiple species, *B. vinsonii* subsp. *berkhoffii* DNA was more frequently amplified directly from blood ($P = .0136$, logistic regression). *Bartonella* infection was

Table 1. Serology and PCR results from 61 *Bartonella*-infected dogs from which blood and other fluid samples were tested by BAPGM enrichment culture.

	Bartonella IFA Results (Number Positive Results/Number Tested)		*Bartonella* PCR Results (Number Positive Results/Number Tested)			
Bartonella Species Detected in 61 Infected Dogs, n (%)	*Bh*	*Bvb*	BAPGM Culture	Plate Isolate after BAPGM Culture	BAPGM and Plate Culture Combined	Only Detected by BAPGM*
Bh, 30 (49%)	7/19 (37%)	4/17 (24%)	14/30 (47%)	5/30 (17%)	19/30 (63%)	15/30 (50%)
Bvb, 17 (28%)	4/10 (40%)	4/10 (40%)	6/17 (35%)	6/17 (35%)	10/17 (59%)	9/17 (53%)
Bh + *Bvb*, 7 (11%)	0/2 (0%)	0/2 (0%)	6/7 (86%)	3/7 (43%)	6/7 (86%)	5/7 (71%)
Bk, 2 (3 %)	NP	NP	1/2 (50%)	0/2 (0%)	1/2 (50%)	1/2 (50%)
Bvl, 2 (3%)	0/1 (0%)	NP	0/2 (0%)	2/2 (100%)	2/2 (100%)	2/2 (100%)
B. bovis, 1 (1.6%)	NP	NP	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
Bvb+ *Bk*, 1 (1.6%)	NP	NP	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
Bvb + *Bvl*, 1 (1.6%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)
Total, 61 (100%)	11/33 (33%)	8/30 (27%)	29/61 (48%)	16/61 (26%)	40/61 (66%)	32/61 (53%)

*BAPGM, *Bartonella* α-*Proteobacteria* growth medium; *B. bovis*, *Bartonella bovis*; *Bh*, *Bartonella henselae*; *Bk*, *Bartonella koehlerae*; *Bvb*, *Bartonella vinsonii* subsp. *berkhoffii*; *Bvl*, *Bartonella volans-like*; IFA, immunofluorescent antibody assays.

not statistically associated with illness duration or concurrent antibiotic and corticosteroid therapy.

Overall, concurrently tested *B. henselae* and *B. vinsonii* subsp. *berkhoffii* serology results were available for 30/61 (49%) *Bartonella*-infected dogs. Only 7/19 (36.8%) *B. henselae* and 4/10 (40%) *B. vinsonii* subsp. *berkhoffii* infected dogs for which sera were available were seroreactive to *B. henselae* and *B. vinsonii* subsp. *berkhoffii* antigens (IFA reciprocal titers of 64 or greater), respectively.

There was no statistical agreement between serology and PCR for *B. henselae* ($P = .034$, McNemar test); however, agreement was detected between *B. vinsonii* subsp. *berkhoffii* PCR and serology ($P = .267$, McNemar test). Serum was not submitted from any coinfected dog.

Discussion

This retrospective study provides molecular and microbiological evidence that sick dogs can be infected with a diversity of *Bartonella* species, that enrichment culture and subculture, followed by PCR amplification, enhances molecular diagnostic sensitivity, and that IFA serology is diagnostically insensitive. Overall, 9.2% (61 of 663) of the dogs tested were infected with 1 or 2 *Bartonella* spp., with coinfection documented in 14.7% of the infected dogs. *B. henselae* and *B. vinsonii* subsp. *berkhoffii* were the most frequently amplified *Bartonella* sequences, accounting for 49.2 and 27.9%, respectively, whereas less frequently detected species included *B. koehlerae*, *Bartonella volans-like*, and *B. bovis*. The results of this study cannot be generalized to all sick dog populations, because the authors and other researchers continue to attempt to define the spectrum of disease associated with canine bartonellosis. In conjunction with this process, diagnostic use of the BAPGM platform in this study often was attempted for complex referral cases and for dogs that failed antibiotic therapy directed at known vector-borne pathogens. Future studies should compare serology and BAPGM platform results among healthy dogs and dogs with defined clinical, hematological, or biochemical entry criteria in conjunction with prospectively selected control populations.

Nearly 50% of dogs in this study were being treated with antibiotics at the time of sample collection. As with other bacterial culture approaches, antibiotic therapy may have decreased diagnostic sensitivity of the BAPGM platform, particularly the postenrichment steps. However, *Bartonella* DNA frequently was detected despite antibiotic therapy, which may reflect a high degree of treatment failure, which has been suspected clinically. *Bartonella* resistance genes recently have been delineated,[16] however, the extent to which resistance, failure of antibiotics to achieve adequate intracellular concentrations, or other factors contribute to antibiotic treatment failures has not yet been determined.[16] If canine bartonellosis is suspected, the results of this study suggest that a microbiological diagnosis might be achieved, despite concurrent antibiotic therapy. Four dogs (6.6%) were being treated with corticosteroids, but the doses prescribed (ie, anti-inflammatory versus immunosuppressive) were not available for all patients. There was no statistical association with enhanced PCR detection; however, few dogs were treated with corticosteroids in this study and therefore, whether steroid therapy might facilitate molecular diagnosis of bartonellosis remains uncertain.

Because of increased awareness among veterinarians and physicians and improvements in diagnostic techniques, infections associated with a diversity of *Bartonella* spp. are being documented more frequently. In the last decade, at least 8 *Bartonella* species, including *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, *B. koehlerae*, *Bartonella clarridgeiae*, *Bartonella elizabethae*, *Bartonella washoensis*, *B. quintana*, and *Bartonella rochalimae* have been implicated as canine pathogens.[1–3] In 2004, *B. henselae*, which for many years was thought to infect cats, but not dogs, was isolated for the first time from a dog from Gabon.[17] Before successful isolation of the organism, *B. henselae* DNA was amplified and sequenced from lymph nodes (generalized granulomatous lymphadenitis),[18–20] liver tissue (granulomatous hepatitis), and from a dog with peliosis hepatis.[19] In this study, *B. henselae* was the most frequently detected *Bartonella* sp., suggesting that increased diagnostic and research attention should be focused on *B. henselae* infection in dogs.

In the context of less frequently detected species (9.2% of the cases in this study), *B. elizabethae* DNA was amplified and sequenced from the blood of a dog with chronic weight loss and sudden death and from the lymph node of a Golden Retriever.[8,20] Recently Kosoy and colleagues identified a spectrum of rodent-associated *Bartonella* species, including *B. elizabethae*, in 31.3% (60/192) of stray dogs in Thailand.[21] *B. washoensis* infection was diagnosed in a dog with mitral valve endocarditis,[22] and *B. clarridgeiae* was associated with endocarditis[22,23] and lymphocytic hepatitis.[19] *B. quintana* also has been identified in 2 dogs with endocarditis.[24] Most recently, *B. rochalimae* was isolated from 3 domestic dogs and from 22 gray foxes (a presumed wildlife reservoir in California),[25] and from a California dog with endocarditis.[26]

Our laboratory has recently reported DNA carryover during the postmortem or histopathological processing of animal tissues,[27] and the possibility of DNA carryover should be considered when interpreting historical reports in which a *Bartonella* sp. was amplified from processed paraffin-embedded tissues. In addition, fresh frozen tissues or strict measures to avoid DNA carryover should be incorporated in all future *Bartonella* studies involving paraffin-embedded tissues. Clinical samples such as blood and diagnostic fluid samples should be collected aseptically to avoid contamination with rapidly growing bacteria, which could negate detection of *Bartonella* spp. during the enrichment process.

Based on serologic evidence, exposure to *B. henselae* has been reported in 3.0% (3/100) of dogs in the United Kingdom,[28] 7.7% (4/52) of dogs in Japan,[29] and 27.2% (82/301) of sick dogs in North Carolina.[30] In this study, infection with *B. henselae* was found more frequently in sick pet dogs than *B. vinsonii* subsp. *berkhoffii* (49.2% versus 27.9%). In comparison, *B. vinsonii* subsp. *berkhoffii* antibodies were reported in 10% (4/40) of dogs from Israel[31] and in 38% (19/49) of dogs from Thailand, where tick transmission of *B. vinsonii* subsp. *berkhoffii* was suspected.[32,33] Based on the results of this study, it is likely that serology will underestimate the prevalence of

Bartonella infection in epidemiological studies and in clinical cases involving dogs.

This study also provides initial documentation for infection with a novel *Bartonella* sp. in dogs from the southeastern United States. *Bartonella volans-like* is closely related to *Candidatus B. volans*, which was isolated from flying squirrels in the southeastern United States.[34] We also report *Bartonella koehlerae* bacteremia in 2 dogs from the United States. Aortic valve endocarditis in a dog from Israel represents the only other published report of *B. koehlerae* infection in dogs.[35] Our results, in conjunction with previous case reports, suggest that a diverse spectrum of *Bartonella* spp. are capable of infecting pet dogs in the United States. Also, this study provides additional evidence that *Bartonella* spp. DNA can be found in a spectrum of patient sample types.[8,36,37] *Bartonella* DNA was found in blood (EDTA and serum), tissue biopsies, body fluids (including joint and CSF, seroma, or cavitary effusions), lymph node, and bone marrow aspirates. Based on consecutive blood culture data, a relapsing pattern of bacteremia develops in experimentally infected cats,[38] in rodents[37] and, based on sequential BAPGM cultures in our laboratory, most likely in human patients (Breitschwerdt, unpublished data).[39] A relapsing pattern of bacteremia also may occur in infected dogs. For this reason, sampling at a single time point could result in a false negative diagnostic result. Prospective studies are necessary to determine which sample source would be most likely to generate positive diagnostic test results in a given patient and the frequency at which blood cultures should be performed for optimal detection.

By IFA testing, we found that only 25% of *B. henselae* infected dogs and only 50% of the *B. vinsonii* subsp. *berkhoffii* infected dogs were seroreactive. Unfortunately, concurrently obtained serology results were only available for 49% of the *Bartonella*-infected dogs in this study. Similar discrepancies between serology and PCR analyses frequently are observed in chronic intraerythrocytic and occult vector-borne infections, including babesiosis and leishmaniasis.[40,41] Genetic methods, such as PCR amplification of organism-specific DNA sequences, provide a more sensitive and specific means of detecting acute infections. Seronegativity may reflect an inadequate time interval after infection for development of a diagnostically detectable antibody response, because almost 50% of the dogs diagnosed with bartonellosis in this study had a duration of clinical signs <1 month. After experimental infection, 6/6 dogs seroconverted by day 7 postinfection, but the degree and rapidity of antibody production was variable.[42] Unfortunately, data regarding the canine humoral response after natural infection are lacking. Until recently, serology has been considered the gold standard method for the diagnosis of *Bartonella* spp. infection in dogs and human patients.[1] Because the production of antibodies in infected people against the infecting *Bartonella* spp. is highly variable, serology appears to be diagnostically insensitive in both dogs and human patients.[43–48] In conclusion, as with the diagnosis of many highly fastidious infectious diseases, concurrently obtaining serology, PCR, and optimized enrichment culture results will enhance diagnostic sensitivity.

In summary, this study found that a diversity of *Bartonella* species were isolated or PCR amplified from various diagnostic specimens obtained from 61 sick dogs. In agreement with seroepidemiological studies, sick dogs are more frequently exposed to and infected with *B. henselae*, as compared with *B. vinsonii* subsp. *berkhoffii* or other *Bartonella* spp. Although infrequent, dogs can be infected with *B. koehlerae*, *B. bovis*, or a novel *Bartonella* sp. Use of the BAPGM diagnostic platform will improve the microbiological documentation of *Bartonella* infection in dogs and can support the microbiological detection of novel or infrequently identified *Bartonella* spp. This approach should facilitate future studies that are needed to enhance the comparative medical understanding of this genus of bacteria.

Footnotes

[a] Qiagen Inc, Valencia, CA
[b] Blast version 2.0, http://blast.ncbi.nlm.nih.gov/blast.cgi
[c] Cell-Line/Thermo Scientific, Pittsburgh, PA
[d] JMP, SAS Institute Inc, Cary, NC

Acknowledgments

This research was supported in part by a grant from the American College of Veterinary Internal Medicine Foundation, the Kindy French Foundation, the State of North Carolina, IDEXX Laboratories, and Bayer Corporation. The authors are grateful to Julie M. Bradley for serological testing, Barbara Hegarty for preparation of diagnostic antigens, Tonya Lee for editorial assistance, Dr Philip Kass from UC Davis for statistical advice, and to the numerous veterinarians from throughout the United States who requested diagnostic testing of the dogs reported in this study.

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