Received: 8 February 2021 | Accepted: 20 January 2022

DOI: 10.1111/jvim.16376

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Associations between presence of *Bartonella* species deoxyribonucleic acid and complete blood cell count and serum biochemical changes in client-owned cats

Maggie Williams[1] | **Sangeeta Rao**[1] | **Jennifer Braff**[2] | **Jesse S. Buch**[2] | **Ramaswamy Chandrashekar**[2] | **Michael R. Lappin**[1]

[1]Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado, USA

[2]IDEXX Laboratories, Inc., Westbrook, Maine, USA

Correspondence
Maggie Williams, Department of Clinical Sciences, Colorado State University, 300 West Drake Road, Fort Collins, CO 80523, USA.
Email: maggie.williams@colostate.edu

Abstract

Background: Infection with *Bartonella* species is common in cats but reported effects of bacteremia on laboratory variables differ.

Objectives: Evaluate for associations between *Bartonella* bacteremia and CBC and serum biochemical changes in sick and healthy cats throughout the United States.

Animals: A total of 3964 client-owned cats.

Methods: Retrospective cohort study using submissions to a commercial laboratory between 2011 and 2017. Serum biochemistry and CBC abnormalities (categorized as above or below reference intervals), age, and location (high- or low-risk state for *Ctenocephalides felis*) in presumed healthy and sick cats were evaluated for associations with presence of *Bartonella* spp. DNA, detected by PCR. Univariate and multivariable logistic regression analyses were performed.

Results: *Bartonella* spp. DNA was amplified from 127 (3.2%) of 3964 cats; 126 (99.2%) of 127 were from high flea risk states and 121 (95.3%) of 127 were presumed sick. Fever of unknown origin was the most common PCR panel requested. In the multivariable analysis, neutrophilia, decreased ALP activity, clinical status (presumed sick), and young age (≤2 years) each were positively associated whereas neutropenia and hyperproteinemia both were negatively associated with *Bartonella* spp. bacteremia. Presence of *Bartonella* spp. DNA had no association with test results for other infectious disease agents.

Conclusions and Clinical Importance: In both healthy and sick cats, active *Bartonella* infections had minimal association with clinically relevant laboratory abnormalities. However, based on these results, in areas considered high risk for *C. felis*, active infection with *Bartonella* spp. is a reasonable differential

ABBREVIATIONS: ALP, alkaline phosphatase; ALT, alanine aminotransferase; DNA, deoxyribonucleic acid; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; FUO, fever of unknown origin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; OR, odds ratio; SDMA, symmetric dimethylarginine; Spp., species; WBC, white blood cell.

diagnosis for cats presented with unexplained fever and neutrophilia, particularly if the cat is young.

KEYWORDS
bacteremia, bartonellosis, fever, PCR

1 | INTRODUCTION

Domestic cats are the primary reservoir hosts for *Bartonella henselae*, *B. clarridgeae*, and *B. koehlerae*.[1,2] Exposure to *Ctenocephalides felis*, the primary vector of several *Bartonella* species (spp.) in cats, is an important risk factor for transmission.[1,3,4] Regional differences in seroreactivity and presumed exposure to *Bartonella* spp. coinciding with climate and flea prevalence exist.[5-8]

Bartonella spp. infection in cats is often subclinical, but a variety of clinical conditions including fever, endocarditis, myocarditis, lethargy, lymphadenopathy, anorexia, neurologic disorders, hematuria, and uveitis have been reported.[7,9-13] Various laboratory abnormalities also have been reported including hyperproteinemia, hyperglobulinemia, decreased blood glucose concentrations, lymphocytosis, neutropenia, thrombocytopenia, eosinophilia, and decreased erythrocyte mean corpuscular volume (MCV).[1,10,14-17]

Diagnostic testing methods for *Bartonella* spp. include serology or demonstration of the presence of *Bartonella* spp. or *Bartonella* deoxyribonucleic acid (DNA) by culture, PCR, and histochemistry or immunohistochemistry.[1,18] Serologic testing is of limited utility when assessing for active infection in cats or associations with clinical syndromes, because seroprevalence rates up to 93% have been reported in areas endemic for *C. felis*.[6] Additionally, seroconversion does not always occur and failure to seroconvert may play a role in development of clinical illness.[1,7,9] Determination of active infection in cats therefore relies on detection of *Bartonella* spp. bacteremia by culture or PCR amplification of *Bartonella*-specific DNA, with the latter being used more commonly in clinical practice because final culture results can take weeks to return because of the fastidious and slow-growing nature of this genus.[1,18] However, *Bartonella* spp. bacteremia is possible in apparently clinically healthy cats, and thus culture of the organism or amplification of *Bartonella*-specific DNA by PCR assay does not confirm causation of signs in a sick cat. Furthermore, both intermittent bacteremia and false negative PCR results can occur.[1,18-20]

Testing and treatment for subclinical *Bartonella* spp. infections in cats is not generally recommended by the Center for Disease Control and Prevention and other public health groups because of concerns for increased risk of antimicrobial resistance.[21-24] Knowing the clinical syndromes and laboratory abnormalities most commonly associated with clinical bartonellosis in cats therefore would be beneficial to help clinicians decide when infection with *Bartonella* spp. should be considered as a differential diagnosis in sick cats. Although *Bartonella* spp. infection in cats has been studied extensively, limitations of previous studies include small sample sizes, limited geographical ranges, and use of serology as the diagnostic method.

Our primary objective was to evaluate for associations between the presence of *Bartonella* spp. DNA, determined by real-time PCR, and CBC and serum biochemistry results in a large number of client-owned healthy and presumed sick cats throughout the United States. The secondary objective was to evaluate for associations between *Bartonella* spp. PCR results and age, geographical location, and presence of selected co-infections.

2 | MATERIALS AND METHODS

Ours was a retrospective cohort study comparing cats with *Bartonella* spp. DNA detected by PCR to those in which *Bartonella* spp. DNA was not detected. The medical records database of a commercial laboratory (IDEXX Laboratories, Inc, Westbrook, Maine) was reviewed for submissions from cats between 2011 and 2017 that had blood evaluated by real-time PCR assay that amplifies DNA of *Bartonella* spp. including *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, and *B. quintana* (Bartonella RealPCR test, IDEXX Laboratories, Inc, Westbrook, Maine). Speciation was not performed and, for the purposes of the study, cats that had a positive *Bartonella* spp. PCR were referred to as being *Bartonella*-positive and presumed to have bacteremia. To be included, samples must have had a CBC and serum biochemical panel submitted concurrently to the same laboratory. When available, results from additional infectious disease tests were included. These assays included ELISA for feline immunodeficiency virus (FIV) antibody (FIV Antibody by ELISA, IDEXX Laboratories, Inc, Westbrook, Maine) and feline leukemia virus (FeLV) antigen (FeLV Antigen by ELISA, IDEXX Laboratories, Inc, Westbrook, Maine), and PCR assay results for nucleic acids of FeLV, FIV, *Mycoplasma haemofelis*, "*Candidatus* Mycoplasma haemominutum," "*Candidatus* Mycoplasma turicensis", feline coronavirus, feline calicivirus, feline panleukopenia virus, *Cytauxzoon felis*, *Anaplasma* spp., *Ehrlichia* spp., *Cryptococcus* spp., *Salmonella* spp., and *Toxoplasma* spp. (RealPCR Tests [various], IDEXX Laboratories, Inc, Westbrook, Maine). Cats were considered positive for FeLV or FIV if they tested positive by either PCR or ELISA or both. Submissions were sorted, and if potential duplicates were identified based on age, sex, breed, submitting clinic, and date of submission, the associated CBC and serum biochemistry results were manually reviewed to evaluate for duplicate samples. If the clinicopathologic data differed, the samples were considered to be independent samples. No owner or practice information was shared or included in analysis.

Cats were stratified into groups based on the PCR panel that was ordered: blood donor screening, anemia, *Bartonella* stand-alone request, fever of unknown origin (FUO), vector-borne disease, or neurologic disease (Appendix S1). Clinical status was defined as healthy or presumed sick, with animals tested using the blood donor panel presumed healthy and all others presumed sick.

Other submission information collected included age, breed, and clinic location by state. Cats were classified as young (0-2 years), adult (3-10 years), or older (>10 years) based on a modification of the American Association of Feline Practitioners/American Animal Hospital Association Feline Life Stage Guidelines.[25] The states of origin were divided into high or low risk for exposure to the primary vector for transmission, *C. felis*, as previously described.[5] Low-risk states were defined as Alaska, Arizona, Colorado, Montana, New Mexico, Nevada, and Utah.

Complete blood count variables that were evaluated were hematocrit, mean corpuscular hemoglobin concentration (MCHC), MCV, and counts of total white blood cell (WBC), neutrophils, lymphocytes, platelets, and reticulocytes. When possible, anemic cats were further categorized as having nonregenerative or regenerative anemia, with reticulocyte counts >50 000/μL considered regenerative. Serum biochemical variables that were evaluated included concentrations of total protein, albumin, globulins, total bilirubin, creatinine, symmetric dimethylarginine (SDMA), and glucose, albumin/globulin ratio, and activities of alkaline phosphatase (ALP) and alanine aminotransferase (ALT). For bilirubin and SDMA concentrations, results were classified categorically as being within or above laboratory reference intervals. For all other variables, results were classified categorically as below, within, or above laboratory reference intervals.

2.1 | Statistical analysis

Univariate analysis of the following variables and presence or absence of *Bartonella* spp. DNA were performed: Hematocrit, reticulocyte count, MCV, MCHC, WBC count, absolute neutrophil count, absolute lymphocyte count, platelet count, concentrations of bilirubin, total protein, globulins, albumin, glucose, creatinine, and SDMA, and activities of ALT and ALP. Additional variables evaluated were age, clinic state, concurrent FeLV or FIV infection, concurrent presence of DNA of the other infectious agents, and health status (healthy vs presumed sick).

Categorical data were arranged in contingency tables to evaluate associations between the individual variables and the presence or absence of *Bartonella* spp. DNA as univariate analyses. For all CBC and serum biochemical panel variables, comparisons were made between the stratified categories with increased or decreased results from the reference range compared to those results within reference ranges. Anemic cats also were compared to the combination of cats with normal and increased hematocrits. Odds ratios (ORs) and 95% confidence limits were calculated for the variables with 2 categories. Chi-square and associated *P*-values were reported. If any of the category expected results numbered <5, Fisher's exact test was used to evaluate for significance. Variables that were significantly associated with the outcome at $P < .25$ were selected as eligible for multivariable logistic regression analysis. A backward elimination process was undertaken to retain the variables with $P < .05$ in the final multivariable logistic model. The program SAS v 9.4 (SAS Institute Inc., Cary, North Carolina) was used for all statistical analyses.

3 | RESULTS

During the time period studied, the *Bartonella* spp. PCR assay was performed on 26 205 cats. No duplicate submissions were identified. After excluding those cases without concurrent CBC and serum biochemical panels (n = 22 241), records of 3964 submissions were identified that were eligible for inclusion. Of these, 287 (7.2%) of 3964 were from low-risk states, 3677 (92.8%) of 3964 were from high-risk states, 1282 (32.3%) of 3964 were presumed healthy, and 2682 (67.7%) of 3964 were presumed sick. Age was not reported for 138 cats, 1364 (35.7%) of 3826 were young, 1937 (50.6%) of 3826 were adult, and 525 (13.7%) of 3826 were older.

Deoxyribonucleic acid of *Bartonella* spp. was amplified from 127 (3.2%) of 3964 cats, of which 121 (95.3%) of 127 were presumed sick and 6 (4.7%) of 127 were presumed healthy ($P < .001$). The following categories of PCR panels were selected by referring veterinarians: *Bartonella* stand-alone request (n = 667; 16.8%), anemia (649; 16.4%), FUO (n = 451; 11.4%), neurologic disease (n = 152; 3.8%), vector-borne disease testing (n = 763; 19.3%), and blood donor screening (n = 1282; 32.3%; Table 1; Appendix S1). Fever of unknown origin was the panel most commonly associated with positive PCR results (53/127; 41.7%). Blood donor screening was the panel with the lowest percent of positive PCR results (6/1272; 0.47%), whereas neurologic disease was the panel making up the lowest total number of positive PCR results (3/127; 2.4%).

Samples were submitted from all 48 contiguous states and Hawaii. The largest number of samples was from California (n = 1020), followed by Florida (n = 305), Texas (n = 201), Colorado (n = 185), New York (n = 178), Massachusetts (n = 171), Oregon (n = 166), Georgia (n = 138), Illinois (n = 118), North Carolina (n = 113), Pennsylvania (n = 113), and Ohio (n = 110). All other states had <100 cats represented. Compared to low-risk states, high-risk states submitted more samples (3677/3964; 92.8% vs 287/3964; 7.2%) had higher proportions of presumed sick cats (2567/3677; 69.8% vs 115/287; 40.1%; Chi-square, 106.27; $P < .001$), and higher proportions of cats positive for *Bartonella* spp. DNA (126/3677; 3.4% vs 1/287; 0.4%; $P = .001$).

Of the cats with *Bartonella* spp. DNA amplified from blood, 126 (99.2%) of 127 were from high-risk states, 1 (0.8%) of 127 was from a low-risk state (New Mexico), 68 (53.5%) of 127 were young, 48 (37.8%) of 127 were adult, and 11 (8.7%) of 127 were older. Four (66.7%) of 6 of the positive healthy cats were young. Co-infections were uncommon and no associations were found between PCR

TABLE 1 Results of PCR for DNA of *Bartonella* spp. in client-owned cats, stratified by the PCR panel submitted

PCR panel submitted	PCR negative	PCR positive	Total	% Positive (PCR panel category)	% Positive (total)
Blood donor	1276	6	1282	0.47	4.7
Neurologic	149	3	152	1.97	2.4
Anemia	635	14	649	2.16	11.0
VBD	736	27	763	3.54	21.3
Bartonella alone	643	24	667	3.60	18.9
FUO	398	53	451	11.75	41.7
Total	3837	127	3964	3.20	

Note: Percent (%) positive is % of cats testing positive out of total cats in that PCR panel category or out of the total number of cats testing positive for *Bartonella* spp. DNA. Results are listed in ascending order of % positive (PCR panel category).
Abbreviations: FUO, fever of unknown origin; VBD, vector-borne disease.

TABLE 2 Results of concurrent PCR testing for various infectious agents and *Bartonella* spp. in client-owned cats

		Bartonella spp.			
		Negative	Positive	Total	% positive
Mycoplasma haemofelis	Negative	2155	57	2212	2.6
	Positive	87	0	87	0
"*Candidatus* Mycoplasma haemominutum"	Negative	1991	51	2042	2.5
	Positive	252	6	258	2.3
"*Candidatus* Mycoplasma turicensis"	Negative	2194	55	2249	2.4
	Positive	49	2	51	3.9
Feline coronavirus	Negative	1775	62	1837	3.4
	Positive	48	0	48	0
Calicivirus	Negative	390	53	443	12.0
	Positive	9	0	9	0
Feline panleukopenia virus	Negative	235	35	270	13.0
	Positive	8	0	8	0
Cytauxzoon felis	Negative	3027	100	3127	3.2
	Positive	20	0	20	0
Anaplasma spp.	Negative	3019	99	3118	3.2
	Positive	27	1	28	3.6
Ehrlichia spp.	Negative	3036	100	3136	3.2
	Positive	10	0	10	0
Cryptococcus spp.	Negative	549	56	605	9.3
Salmonella spp.	Negative	242	35	277	12.6
Toxoplasma spp.	Negative	546	55	601	9.2
	Positive	1	1	2	50

Note: Positive result indicates nucleic acid for that agent was amplified. Percent (%) positive indicates the respective percentage of total animals that had positive for *Bartonella* spp. PCR results.

results for *Bartonella* spp. and any other infectious disease agents, including hemoplasmas (Table 2).

3.1 | Multivariable analysis

Eighteen variables that were significantly associated ($P < .25$) with the presence of *Bartonella* spp. DNA in the univariate analysis were eligible for inclusion in the multivariable analysis and are listed in Table 3. Variables that were significant ($P < .05$) in the final model are listed in Table 4. Of these, neutrophilia, decreased ALP activity, clinical status (presumed sick), and young age were significantly associated with the presence of *Bartonella* spp. DNA. Neutropenia and hyperproteinemia were negatively associated with presence of *Bartonella* spp. DNA

TABLE 3 Variables associated ($P < .25$) with the presence of *Bartonella* spp. DNA, detected by PCR, in a univariate analysis and included in a multivariable analysis

Variable	Deviation from reference interval	Number of cats with deviation of variable (number of cats with variable measured)	Odds ratio	95% confidence limits		*P*-value
MCHC	↓	86 (3959)	1.93	0.77	4.84	.16
MCV	↑	201 (3958)	0.30	0.07	1.24	.1
	↓	327 (3958)	1.45	0.84	2.52	.19
Neutrophils	↑	332 (3960)	3.50	2.28	5.37	<.001
	↓	336 (3960)	0.53	0.21	1.32	.17
Reticulocytes	↑	305 (3502)	0.47	0.19	1.15	.1
	↓	126 (3502)	1.90	0.90	3.98	.09
Total WBC count	↑	395 (3964)	2.98	1.95	4.56	<.001
Albumin : globulin ratio	↓	318 (3962)	0.55	0.24	1.27	.16
Albumin	↓	581 (3964)	2.67	1.81	3.94	<.001
ALP	↓	547 (3955)	7.16	4.93	10.39	<.001
ALT	↑	163 (3956)	1.63	0.74	3.58	.22
	↓	435 (3956)	2.59	1.69	3.99	<.001
Creatinine	↓	501 (3958)	2.05	1.34	3.14	<.001
Glucose	↑	313 (3956)	2.62	1.64	4.19	<.001
	↓	273 (3956)	0.49	0.18	1.35	.17
SDMA	↑	336 (1997)	0.37	0.15	0.91	.03
Total bilirubin	↑	423 (3816)	2.16	1.36	3.42	.001
Total protein	↓	354 (3964)	1.91	1.17	3.12	.01
Young vs adult		1364, 1937 (3826)	2.07	1.42	3.01	<.001
Young vs senior		1364, 525 (3826)	2.45	1.29	4.67	.006
High-risk state		3677 (3964)	10.15	1.41	72.87	.02
Presumed sick		2682 (3964)	10.05	4.41	22.87	<.001
Nonregenerative anemia		592 (3853)	1.32	0.84	2.06	.23
Regenerative anemia		207 (3853)	0.15	0.02	1.04	.06

Note: Where applicable, ORs were calculated for animals with values above or below laboratory reference intervals when compared to those with values within reference intervals. For age comparisons, young animals are listed first.
Abbreviations: ↑, increased above reference interval; ↓, decreased below reference interval; WBC, white blood cell.

TABLE 4 Variables that retained at least 1 significant comparison ($P < 0.05$) for association of positive PCR for *Bartonella* spp. DNA in a final multivariable analysis

Variable	Comparison	Odds ratio	95% confidence limits		*P*-value
Neutrophils	↑ vs RI	1.93	1.21	3.09	.006
	↓ vs RI	0.33	0.13	0.83	.02
ALP	↑ vs RI	0.90	0.40	2.03	.8
	↓ vs RI	5.06	3.40	7.52	<.001
Total protein	↑ vs RI	0.22	0.08	0.63	.005
	↓ vs RI	1.40	0.82	2.37	.22
Age	Young vs adult	2.26	1.52	3.36	<.001
	Senior vs adult	0.56	0.28	1.13	.11
	Young vs senior	4.03	2.03	8.02	<.001
Clinical group	Presumed sick vs healthy	8.08	3.50	18.68	<.001

Abbreviation: RI, laboratory reference interval.

4 | DISCUSSION

A number of clinical conditions and clinicopathologic changes have been reported in cats with bartonellosis, with many of these associations being inconsistently documented and most being from studies limited by small sample sizes and use of serology, which does not indicate active infection. Given these factors, knowing when specific testing for *Bartonella* spp. is warranted and when treatment of bacteremic cats is indicated can be confusing. We confirmed previous findings that *Bartonella* spp. bacteremia is more common in areas considered high risk for *C. felis* infestation,[5-8] in young cats,[8] and is commonly associated with fever.[9,10,12,13,26,27] Associations with other clinically relevant clinicopathologic abnormalities in both healthy and presumed ill cats were not identified.

Similar to previous studies,[5-8] geographical location was strongly associated with prevalence of *Bartonella*, with California, Florida, Texas, and New York having both the largest number and proportion of positive samples. Only 1 sample from a low-risk state (New Mexico) was positive; it was from a presumed febrile cat and likely indicates a true positive. Possible explanations for this result include local exposure to *C. felis* or other fleas that can carry *Bartonella* spp. but are more tolerant to of low humidity such as *Pulex* spp.,[28,29] or previous travel. Despite this individual, more presumed sick cats were positive for *Bartonella* in high-risk states (4.7%) compared to low-risk states (0.9%), suggesting that in areas with low infestation rates for *C. felis* and in the absence of previous travel, *Bartonella* is unlikely to be the cause of fever or other clinical signs in sick cats. However, cats in these locations still have the potential for exposure to rodent fleas carrying *Yersinia pestis*[20] and to *Pulex* spp., and thus flea control still is indicated for cats throughout the United States.[21]

In our study, cats <2 years of age were more likely to be positive for *Bartonella* spp. DNA. This finding is consistent with a previous study that showed that cats <6 months of age are more likely to have bacteremia than cats >13 months of age,[8] possibly because of increased flea exposure risk, an immature immune system in kittens, or lack of pre-existing immunity.

The odds of *Bartonella* spp. bacteremia were significantly higher in presumed sick cats than in presumed healthy cats, although in both groups, frequencies were low compared to other studies on seroprevalence and bacteremia.[5,6,8,27] Although bacteremia with *Bartonella* spp. often is self-limiting in cats, prolonged bacteremia can occur as a result of relapse or reinfection.[30] The lower prevalences in the client-owned cats of our study may reflect differences in risk factors such as housing and ectoparasite prevention, although our study was not designed to evaluate these factors. Of the presumed sick cats, the FUO panel submission group had the highest proportion of positive PCR results (11.8%) and made up almost half of all the positive results (41.7%). Assuming that the veterinarians only chose this panel to evaluate febrile cats, this finding is consistent with other studies that have associated fever with bacteremia in cats both experimentally and naturally infected with *B. henselae*.[9,10,12,13,26,27] Alternatively, increased awareness of bartonellosis as a cause of fever in cats may have resulted in type I statistical error. In contrast to PCR assay and culture results, an association between fever and positive serological test results has not been identified,[14,27] which is not unexpected given that bartonellosis in cats is often self-limited by a normal immune response. This finding emphasizes that when *Bartonella* spp. testing is performed in sick cats, it should include PCR or culture and not solely serology.

Although *Bartonella* spp. bacteremia was more common in presumed sick cats, the presence of an association does not indicate causation of disease.[9,27] This conclusion was evidenced by the detection of *Bartonella* spp. DNA from 6 potential blood donor cats (0.5%) that are presumed to have been clinically healthy with subclinical infections. The detection of *Bartonella* spp. DNA in these 6 cats also suggests that the recommendation for year-round ectoparasite prevention in potential blood donors is not always adhered to, and highlights the importance of appropriate blood-borne pathogen testing when screening for donors.[18,31]

No association between anemia of any form and *Bartonella* spp. bacteremia was found and only 28 (22%) of 127 bacteremic cats were anemic. Cats with nonregenerative anemias were more likely to be bacteremic in the univariate analysis, but the difference was small, unlikely clinically relevant, and not retained in the multivariable analysis. Only 1 bacteremic cat had a regenerative anemia, a feature common with hemolytic anemia, and contrary to previous findings, no association between changes in MCV and positive *Bartonella*. spp. PCR results was noted.[16] Despite occasional reports of hemolytic anemia in people and dogs,[32,33] anemia has not been documented in any natural *Bartonella* infections in cats, and only transient anemia has been seen after experimental infection.[9,14,16,20,34,35] Our findings add further support to the hypothesis that in its reservoir host, the intraerythrocytic phase of *Bartonella* spp. is a host evasion mechanism that evolved to potentiate ingestion by the flea vector and that in most instances, testing for *Bartonella* infection is not warranted in anemic cats.

We found no association between *Bartonella* bacteremia and hyperglobulinemia, contrary to a previous study in which seropositive cats were more likely to be hyperglobulinemic.[15] The lack of association in our study may be a result of acute infections that occurred before seroconversion and development of hyperglobulinemia. Alternatively, the lack of association reported here may indicate that the nonspecific finding of hyperglobulinemia is not necessarily associated with *Bartonella* infection. An increase in total protein concentration was significantly associated with decreased odds of *Bartonella* bacteremia, but given the association with total protein concentration only, this finding may simply be a reflection of hydration status.

A novel finding in our study was that cats with ALP activity below reference intervals were 5.1× more likely to be bacteremic with *Bartonella* spp. than those with normal ALP activity, and 50% of bacteremic cats had ALP activities below the reference interval compared to 13% of negative cats. However, the positive predictive value of low ALP activity was only 12%. The cause and clinical relevance of the association with lower ALP activity is unknown. Alkaline phosphatases are a group of enzymes that catalyze the hydrolysis of monophosphate esters in the presence of zinc and magnesium. In cats, the

predominant isoform of ALP comes from the liver and has a short half-life of approximately 6 hours.[36] In veterinary medicine, low ALP activities generally are not believed to be clinically relevant findings, but in people they have been associated with malnutrition, recent heart surgery, magnesium or zinc deficiencies, hypothyroidism, anemias, and myelogenous leukemias.[37,38] In the absence of other clinical or clinicopathologic abnormalities, the presence of low ALP activity in a cat is unlikely to be of clinical or diagnostic relevance, but further evaluation is warranted.

In our study, *Bartonella* spp. bacteremia was positively associated with neutrophilia and negatively associated with neutropenia. Neutrophilia is a common finding in stressed or sick animals and the entire clinical picture should be taken into account before testing for *Bartonella*. Of all cats tested for *Bartonella* spp. (n = 3960), neutropenia (n = 336) was as common as neutrophilia (n = 332) but had a negative predictive value of 98.5%, suggesting that if neutropenia is detected in a sick cat, etiologies other than *Bartonella* should be considered.

Our study did not find any associations among increased liver enzyme activities or hyperbilirubinemia and *Bartonella* spp. bacteremia, which is consistent with previous studies that have found no association between peliosis hepatis or liver dysfunction in cats with bartonellosis.[39,40] Whether or not lymphocytic, granulomatous, or neutrophilic hepatitis occur without concurrent serum biochemistry changes is unknown in the cats studied here.[10,12]

No association between presence of *Bartonella* spp. DNA and coinfection with any of the infectious agents evaluated was found, but this finding should be interpreted cautiously because there were a limited number of positive tests results for other infectious disease agents.

Changes in the estimates and significance of some of the variables were found between the univariate and multivariable analysis in our study. For example, hyperproteinemia was not significantly associated with the presence of *Bartonella* spp. in the univariate analysis but was in the multivariable analysis. Step-wise multivariable analysis showed that there were confounding effects of ALP and clinical status on hyperproteinemia because of their effects on estimates and significance. Multivariable analysis takes into account the potential confounding effects of the variables on each other. However, confounding resulting from other unmeasured variables is possible and is a limitation of our study and its retrospective nature because confounding factors could result in over or under-estimation of observed associations identified in our study. Additionally, the final multivariable analysis had fewer subjects because of missing data, which also would have had an impact on the results. Nonetheless, the associations found in our study with geographical location, young age, and clinical status support previously documented associations between *Bartonella* spp. infection in cats with *C. felis* infestations, age, and transient development of fever.

Our study had several additional limitations, predominantly because of its retrospective nature, which precludes knowing the clinical status of patients including important variables such as temperature, concurrent illnesses, previous treatments, length of illness, exposure risks of presumed sick cats, and outcomes. Signalment information such as age and sex relied on the submitting clinic reporting accurate information, and assumptions on clinical status and exposure risk were made when stratifying cats. Clinical status was inferred from the panel requested and although we believe that assuming cats being tested for infectious diseases are clinically ill is valid, some cats tested may have been healthy. For example, although not currently recommended by veterinary or human health associations,[21,23,24] instances exist in which veterinarians or physicians may choose to screen healthy cats in contact with humans who are immunosuppressed or have bartonellosis. It also was assumed that the PCR panel ordered represented the reason for testing a sick cat, but other factors such as economics or screening for specific infectious agents included in the panels can influence which panels veterinarians choose. Each sample in our study was considered an independent sample. However, it is possible that some cats were tested on more than 1 occasion, and therefore a single animal may have had multiple samples included in the analysis. This situation may have occurred because of serial monitoring in blood donor cats or for various reasons in unwell cats. These samples still were considered to be independent from each other because our study was intended to evaluate for associations between bacteremia and clinicopathologic changes at a single timepoint. Submissions with matching ages, sexes, submitting clinics, and submission dates also were included if their CBC and serum biochemical results were different because some clinics send samples in batches, particularly for blood donor screening programs, in which dates of birth are estimated based on the patients' age in years and the current date at the time of submission. Alternatively, an unwell cat may have had samples collected before and after treatment was instituted, but submitted together to minimize shipping costs. Another assumption made was that exposure risk for *Bartonella* spp. infection was based on risk of flea exposure in the state from which a sample was submitted. The low number of positive results in cats designated to be from low-risk states supports this stratification, despite previous travel and residences not being taken into account. Not knowing the length of time of illness is another important limitation, particularly when evaluating for associations with hyperglobulinemia, which is unlikely to be present during the acute phase of infection. Species of *Bartonella* may have different pathogenicities,[17] which was not evaluated in our study. The total number of *Bartonella* spp. positive cats included in the multivariable analysis was only 127 and, in addition to confounding, it is possible that certain clinicopathologic abnormalities were missed as a result of type II statistical error or falsely identified as a result of type I statistical error. Our study was designed to assess for associations between clinicopathologic abnormalities on routine blood test results and the presence of *Bartonella* spp. bacteremia, as detected by PCR, to help understand when testing for *Bartonella* spp. is warranted in the diagnostic evaluation of ill cats. Our results cannot be used to definitely

prove *Bartonella* spp. infection as the cause of clinical signs, or CBC and serum biochemical abnormalities.

In conclusion, in both healthy and presumed ill cats, few clinically relevant clinicopathologic abnormalities were associated with *Bartonella* spp. bacteremia. We did not find evidence to support *Bartonella* spp. testing for routine evaluation of anemia, hypoglycemia, thrombocytopenia, neutropenia, or lymphocytosis in cats. However, in areas considered high risk for *C. felis*, active infection with *Bartonella* spp. is a reasonable differential diagnosis for cats presented for evaluation of unexplained fever and neutrophilia, particularly if the cat is young.

ACKNOWLEDGEMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

IDEXX Laboratories provided the data and assisted in editing the final document. Three of the authors (Drs. Braff, Buch, and Chandrashekar) are employees of IDEXX Laboratories, but were not involved in the data collection, entry, or analysis. None of the authors from Colorado State University were directly compensated for this work in any way.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Maggie Williams https://orcid.org/0000-0002-1277-3946
Michael R. Lappin https://orcid.org/0000-0003-1605-9667

REFERENCES

1. Álvarez-Fernández A, Breitschwerdt EB, Solano-Gallego L. Bartonella infections in cats and dogs including zoonotic aspects. *Parasit Vectors*. 2018;11(1):624.
2. Breitschwerdt EB. Feline bartonellosis and cat scratch disease. *Vet Immunol Immunopathol*. 2008;123(1–2):167-171.
3. Lappin MR, Griffin B, Brunt J, et al. Prevalence of *Bartonella* species, haemoplasma species, *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Neorickettsia risticii* DNA in the blood of cats and their fleas in the United States. *J Feline Med Surg*. 2006;8(2):85-90.
4. Barrs VR, Beatty JA, Wilson BJ, et al. Prevalence of Bartonella species, rickettsia felis, haemoplasmas and the Ehrlichia group in the blood of cats and fleas in eastern Australia. *Aust Vet J*. 2010;88(5):160-165.
5. Jameson P, Greene C, Regnery R, et al. Prevalence of Bartonella henselae antibodies in pet cats throughout regions of North America. *J Infect Dis*. 1995;172(4):1145-1149.
6. Nutter FB, Dubey JP, Levine JF, Breitschwerdt EB, Ford RB, Stoskopf MK. Seroprevalences of antibodies against Bartonella henselae and Toxoplasma gondii and fecal shedding of Cryptosporidium spp, giardia spp, and Toxocara cati in feral and pet domestic cats. *J Am Vet Med Assoc*. 2004;225(9):1394-1398.
7. Lappin MR, Hawley J. Presence of Bartonella species and Rickettsia species DNA in the blood, oral cavity, skin and claw beds of cats in the United States: Bartonella DNA in blood and skin of cats. *Vet Dermatol*. 2009;20(5–6):509-514.
8. Guptill L, Wu C-C, HogenEsch H, et al. Prevalence, risk factors, and genetic diversity of Bartonella henselae infections in pet cats in four regions of the United States. *J Clin Microbiol*. 2004;42(2):652-659.
9. Bradbury CA, Lappin MR. Evaluation of topical application of 10% imidacloprid–1% moxidectin to prevent *Bartonella henselae* transmission from cat fleas. *J Am Vet Med Assoc*. 2010;236(8):869-873.
10. Kordick DL, Brown TT, Shin K, Breitschwerdt EB. Clinical and pathologic evaluation of chronic Bartonella henselae or Bartonella clarridgeiae infection in cats. *J Clin Microbiol*. 1999;37(5):1536-1547.
11. Lappin MR, Kordick DL, Breitschwerdt EB. Bartonella spp antibodies and DNA in aqueous humour of cats. *J Feline Med Surg*. 2000;2(1):61-68.
12. Guptill L, Slater L, Wu CC, et al. Experimental infection of young specific pathogen-free cats with Bartonella henselae. *J Infect Dis*. 1997;176(1):206-216.
13. O'Reilly KL, Bauer RW, Freeland RL, et al. Acute clinical disease in cats following infection with a pathogenic strain of Bartonella henselae (LSU16). *Infect Immun*. 1999;67(6):3066-3072.
14. Breitschwerdt EB, Levine JF, Radulovic S, et al. Bartonella henselae and Rickettsia seroreactivity in a sick cat population from North Carolina. *Int J Appl Vet Res*. 2005;3(4):287-402.
15. Whittemore JC, Hawley JR, Radecki SV, Steinberg JD, Lappin MR. Bartonella species antibodies and hyperglobulinemia in privately owned cats. *J Vet Intern Med*. 2012;26(3):639-644.
16. Müller A, Walker R, Bittencourt P, et al. Prevalence, hematological findings and genetic diversity of *Bartonella* spp. in domestic cats from Valdivia, Southern Chile. *Parasitology*. 2017;144(6):773-782.
17. Breitschwerdt EB, Broadhurst JJ, Cherry NA. Bartonella henselae as a cause of acute-onset febrile illness in cats. *JFMS Open Rep*. 2015;1(2):205511691560045.
18. Wardrop K, Birkenheuer A, Blais M, et al. Update on canine and feline blood donor screening for blood-borne pathogens. *J Vet Intern Med*. 2016;30(1):15-35.
19. Breitschwerdt EB. Bartonellosis, one health and all creatures great and small. *Vet Dermatol*. 2017;28(1):96-e21.
20. Kordick DL, Breitschwerdt EB. Relapsing bacteremia after blood transmission of Bartonella henselae to cats. *Am J Vet Res*. 1997;58(5):492-497.
21. Lappin MR, Elston T, Evans L, et al. 2019 AAFP feline zoonoses guidelines. *J Feline Med Surg*. 2019;21(11):1008-1021.
22. Biswas S, Maggi RG, Papich MG, Keil D, Breitschwerdt EB. Comparative activity of pradofloxacin, enrofloxacin, and azithromycin against Bartonella henselae isolates collected from cats and a human. *J Clin Microbiol*. 2010;48(2):617-618.
23. Masur H, Brooks JT, Benson CA, et al. Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: updated guidelines from the Centers for Disease Control and Prevention, National Institutes of Health, and HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis*. 2014;58(9):1308-1311.
24. Brunt J, Guptill L, Kordick DL, Kudrak S, Lappin MR, American Association of Feline Practitioners., Academy of Feline Medicine Advisory Panel. American Association of Feline Practitioners 2006 Panel report on diagnosis, treatment, and prevention of Bartonella spp. infections. *J Feline Med Surg*. 2006;8(4):213-226.
25. Vogt AH, Rodan I, Brown M, et al. AAFP–AAHA Feline life stage guidelines. *J Am Anim Hosp Assoc*. 2010;46:16-85.
26. Mikolajczyk MG, O'Reilly KL. Clinical disease in kittens inoculated with a pathogenic strain of Bartonella henselae. *Am J Vet Res*. 2000;61(4):375-379.

27. Lappin MR, Breitschwerdt E, Brewer M, Hawley J, Hegarty B, Radecki S. Prevalence of *Bartonella* species antibodies and *Bartonella* species DNA in the blood of cats with and without fever. *J Feline Med Surg*. 2009;11(2):141-148.
28. Fenimore A, Varanat M, Maggi R, Schultheiss P, Breitschwerdt E, Lappin MR. Bartonella spp. DNA in cardiac tissues from dogs in Colorado and Wyoming. *J Vet Intern Med*. 2011;25(3):613-616.
29. Yore K, DiGangi B, Brewer M, Balakrishnan N, Breitschwerdt EB, Lappin M. Flea species infesting dogs in Florida and Bartonella spp. prevalence rates. *Vet Parasitol*. 2014;199(3):225-229.
30. Arvand M, Viezens J, Berghoff J. Prolonged Bartonella henselae bacteremia caused by reinfection in cats. *Emerg Infect Dis*. 2008;14(1):152-154.
31. Hackett TB, Jensen WA, Lehman TL, et al. Prevalence of DNA of *mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum,' *Anaplasma phagocytophilum*, and species of *Bartonella*, *Neorickettsia*, and *Ehrlichia* in cats used as blood donors in the United States. *J Am Vet Med Assoc*. 2006;229(5):700-705.
32. Breitschwerdt EB, Blann KR, Stebbins ME, et al. Clinicopathological abnormalities and treatment response in 24 dogs seroreactive to *Bartonella vinsonii* (*berkhoffii*) antigens. *J Am Anim Hosp Assoc*. 2004;40(2):92-101.
33. Van Audenhove A, Verhoef G, Peetermans WE, et al. Autoimmune haemolytic anaemia triggered by Bartonella henselae infection: a case report. *Br J Haematol*. 2001;115(4):924-925.
34. Ishak A, Radecki S, Lappin M. Prevalence of Mycoplasma haemofelis, 'Candidatus Mycoplasma haemominutum', Bartonella species, Ehrlichia species, and Anaplasma phagocytophilum DNA in the blood of cats with anemia. *J Feline Med Surg*. 2007;9(1):1-7.
35. Kordick DL, Breitschwerdt EB. Intraerythrocytic presence of Bartonella henselae. *J Clin Microbiol*. 1995;33(6):1655-1656.
36. Fernandez NJ, Kidney BA. Alkaline phosphatase: beyond the liver. *Vet Clin Pathol*. 2007;36(3):223-233.
37. Sharma U, Pal D, Prasad R. Alkaline phosphatase: an overview. *Ind J Clin Biochem*. 2014;29(3):269-278.
38. Lum G. Significance of low serum alkaline phosphatase activity in a predominantly adult male population. *Clin Chem*. 1995;41(4):515-518.
39. Brown PJ, Henderson JP, Galloway P, O'Dair H, Wyatt JM. Peliosis hepatis and telangiectasis in 18 cats. *J Small Anim Pract*. 1994;35(2):73-77.
40. Buchmann AU, Kempf VA, Kershaw O, et al. Peliosis hepatis in cats is not associated with Bartonella henselae infections. *Vet Pathol*. 2010;47(1):163-166.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Williams M, Rao S, Braff J, Buch JS, Chandrashekar R, Lappin MR. Associations between presence of *Bartonella* species deoxyribonucleic acid and complete blood cell count and serum biochemical changes in client-owned cats. *J Vet Intern Med*. 2022;36(2):532-540. doi:10.1111/jvim.16376