Bartonella henselae and *Rickettsia* Seroreactivity in a Sick Cat Population from North Carolina

Edward B. Breitschwerdt, DVM, DACVIM[1]
Jay F. Levine, DVM, PhD[2]
Suzana Radulovic, PhD[3]
Sheila B. Hanby, DVM[1]
Dorsey L. Kordick, PhD[1]
Krista M.D. La Perle, DVM[2]

[1]*Departments of Clinical Sciences*
College of Veterinary Medicine
North Carolina State University
Raleigh, North Carolina

[2]*Population Health and Pathobiology*
College of Veterinary Medicine
North Carolina State University
Raleigh, North Carolina

[3]*Department of Microbiology and Immunology*
University of Maryland School of Medicine
Baltimore, Maryland

KEY WORDS: cats, *Bartonella*, *Rickettsia*, fleas

ABSTRACT

The objective of this study was to evaluate demographic and clinicopathologic factors that may be associated with seroreactivity to *Bartonella henselae* or *Rickettsia* antigens in cats. Medical records of 436 sick cats were reviewed. Of 436 cat sera tested for reactivity to *B. henselae* and *R. typhi* antigens by indirect fluorescent antibody, 112 (26%) were reactive to *B. henselae* antigens, 93 (21%) were reactive to *R. typhi* antigens, and 31 (7%) were reactive to both test antigens. *Bartonella henselae* seroreactors were more likely to be male domestic long-hair or domestic short-hair cats that were allowed access to outdoor areas. Lymphocytosis was associated with *B. henselae* antibodies. Cats with lymphadenopathy and elevated packed cell volume were more likely to have *Rickettsia* antibodies.

INTRODUCTION

During the past decade, 2 novel organisms, *Bartonella henselae*[1,2] and *Rickettsia felis*,[3] have been characterized as zoonotic flea-transmitted pathogens. Both organisms have been isolated from *Ctenocephalides felis*, the cat flea.[4-6] Vector competence has been demonstrated experimentally for cat-to-cat transmission of *B. henselae* and *R. felis* by fleas.[3,7] Bacteriologic and serologic evidence suggests that cats are a primary reservoir for *B. henselae*, which can persist in the vasculature for prolonged periods with a relapsing pattern of bacteremia.[1,8] The extent to which cats or other animals, such as opossums, which are frequently infested with *C. felis*, serve as a reservoir for *R. felis* is currently being studied.[3] Flea infestations of household pets and peridomestic animals most

probably contribute to the maintenance of these organisms in close proximity to household environments.

Both *B. henselae* and *R. felis* cause febrile illness in people.[9,10] The spectrum of human disease associated with *B. henselae* includes bacillary angiomatosis, vasoproliferative lesions in the liver or spleen (peliosis hepatis and peliosis splenitis), granulomatous hepatosplenic syndrome, endocarditis, lymphadenopathy (cat scratch disease), osteolytic lesions, pulmonary granulomas, a spectrum of ophthalmic abnormalities, including neuroretinitis, neurologic dysfunction, and fever and bacteremia.[2,9,11] *Rickettsia felis* was first recognized as a cause of febrile illness in a human patient from Texas.[10] Subsequently, typhus-like illness was reported in people residing in Central and South America and Europe.[12-15] Recent, molecular detection of several *Bartonella* species, *R. felis*, and *Wolbachia pipientis* in cat fleas from France suggests that cat fleas may be more important vectors for animal and human diseases than previously reported.[16]

The pathogenicity of *B. henselae* and *R. felis* in cats has not been clearly established. Due to the high prevalence of bacteremia in selected healthy cat populations, *B. henselae* was initially presumed to be minimally or non-pathogenic in cats. Evolving clinical, epidemiological, and experimental infection data indicate that this is not true. Transient lethargy, fever, neurologic dysfunction, reproductive failure, and mild anemia have been reported in cats experimentally infected with *B. henselae* by both blood transfusion and subcutaneous inoculation of culture grown organisms.[1,8,17-21] The role of *R. felis* as a pathogen in cats or the extent to which co-infection with *B. henselae* and *R. felis* might increase the pathogenicity of one or both organisms has not been established. The purpose of this investigation was to perform a retrospective case-reference study of the clinicopathological and epidemiological features of illness in cats that were seroreactive to *B. henselae* and/or *Rickettsia*. In this manner, we attempted to determine if there was any potential association between exposure to these organisms and specific disease manifestations in cats.

MATERIALS AND METHODS

Serosurvey Selection

Between January 25, 1993, and May 31, 1994, serum samples were collected from 436 cats that were examined at the North Carolina State University Veterinary Teaching Hospital (NCSU-VTH) for a variety of illnesses. Serum samples were obtained on a daily basis and stored at –20˚C until assayed.

Indirect Fluorescent Antibody (IFA) Assay

At North Carolina State University College of Veterinary Medicine (NCSU-CVM), *B. henselae* cultured in Vero cells was harvested 3 days post-infection. Antigen for IFA assay was prepared by pelleting the infected cells followed by resuspension in phosphate-buffered saline (PBS). Five microliters of antigen were applied to 24-well Teflon-coated slides, air dried, fixed in acetone for 10 minutes, air dried, and stored at –20˚C until used. Ten microliters of 5% non-fat dry milk in PBS were added to each well and incubated for 30 minutes and then air dried. Cat sera were diluted from 1:16 to 1:1024 in 96-well microtiter plates with 0.5% bovine serum albumin (BSA) in PBS. Ten microliters of diluted serum were applied to each well. Slides were incubated for 30 minutes at 37˚C and then washed with PBS while shaking for 30 minutes. Fluorescein-conjugated goat anti-feline immunoglobulin G (Cappel anti-feline IgG, heavy and light chain specific, Organon Teknika Corp, Durham, NC) was diluted 1:200 in 0.5% BSA-PBS and applied to each well. Slides were incubated for 30 minutes at 37˚C and then washed twice in PBS for 15 minutes while shaking. Slides were examined by fluorescence microscopy at 40× magnification (Zeiss, Germany), as previously described.[8] The *B. henselae* positive control sample was obtained from a

culture-positive, naturally infected cat from North Carolina with a reciprocal titer of 2048. The negative control sample was obtained from a specific-antigen–free (SPF) cat that was non-seroreactive to *B. henselae* antigens on repeated occasions. Cats were classified as *B. henselae* seroreactive when reciprocal titers were 64 or greater.

Serum samples were initially screened for reactivity against *R. typhi* Wilmington strain at a 1:64 dilution, using fluorescein-labeled goat anti-cat IgG (Kirkegaard and Perry, Gaithersburg, MD) at a 1:50 dilution. Those samples positive at a reciprocal titer of 64 were then titrated out to a 1:2048 dilution endpoint. Only samples reactive at a reciprocal titer of 64 and above were deemed positive. For the *R. typhi* IFA assay, ambiguous slides were read by several investigators, and a consensus was required for assignation to the positive cohort.

Definitive Epitope-Blocking Enzyme-Linked Immunosorbent Assay (DEB-ELISA)

A portion of each serum sample was sent by overnight express mail to the University of Maryland Medical School for testing against typhus group rickettsiae. Samples with a *R. typhi* reciprocal titer of 64 or higher (n = 49) were subjected to modified DEB-ELISA.[22] The DEB-ELISA was performed to determine if reactivity was directed against *R. typhi*, as opposed to other strains of *Rickettsia*. Briefly, 96-well plates were coated with *R. typhi* antigen diluted 1:1280 in PBS, and cat sera were applied at a 1:4 dilution and allowed to incubate at 37˚C for 1 hour. After washing, wells received a monoclonal antibody reacting with the *R. typhi* 120 kDa surface protein antigen (SPA) at a 1:5000 dilution for 1 hour at 37˚C. After washing, wells were incubated with peroxidase-labeled goat anti-mouse IgG/IgM (Kirkegaard and Perry, Gaithersburg, MD) at 1:1000 for 1 hour at 37˚C, washed, and then incubated with ABTS/H_2O_2 as substrate for 15 minutes at 37˚C. Plates were read on a Bio-Rad model 2550 EIA reader (Hercules, CA) at 405 nm. The presence of a color reaction indicated that the sample was seronegative for *R. typhi* (ie, the *R. typhi*-specific monoclonal antibody did not block the reaction). Samples with an optical density reading 3 standard deviations above negative controls (SPF cat IFA negative serum) were considered positive for exposure to related *Rickettsia*. As a positive control, we used cat serum from New York, which we have previously shown to be *R. typhi* seroreactive at a reciprocal titer of 2048 by IFA. Three separate DEB-ELISA assays were performed on the 49 samples that were seroreactive to *R. typhi* antigens. In addition, 10 *R. typhi* IFA seroreactive samples were selected at random and tested by IFA against *R. rickettsii* (Sheila Smith strain) antigens.

Medical Data Collection

The age, breed, gender, weight (kg), environment (indoor/outdoor), and month and year of serum sample collection were summarized from the medical record. Age was determined by subtracting the date of birth from the year the blood sample was collected. Cat breeds were recorded as domestic shorthair (DSH), domestic longhair (DLH), or purebreds. Gender was classified as intact male, neutered male, intact female, or spayed female. Environmental status was designated as indoor exclusively, outdoor exclusively, or indoor/outdoor. A cat with any evidence of being outdoors was assigned the designation indoor/outdoor. Temperature, pulse (beats per minute), and respiration (breaths per minute) measurements were recorded for the day of serum sample collection or the day of initial physical examination, if the 2 events did not coincide exactly.

Medical records were reviewed specifically for presence of lymphadenopathy, seizures, non-specific central nervous system dysfunction, lymphocytic plasmacytic hepatitis, granulomatous hepatitis, granulomatous splenitis, lymphocytic nephritis, interstitial nephritis, thromboembolism,

hypertrophic cardiomyopathy, and ocular abnormalities. Other clinical disorders diagnosed in the cats at the time of sample collection were recorded. Some data was unavailable for a small percentage of cats because the information was either recorded as unknown or was not documented in the medical record.

Values derived from complete blood counts (CBC), including differential cell counts, serum biochemical profiles, and urinalyses, were recorded for each cat either on the date of sample collection or on the next closest day within the same hospitalization period (all tests were performed by the NCSU-VTH Clinical Pathology Laboratory). Complete blood counts were performed on EDTA anti-coagulated samples using an automated cell counter (Baker 9010+, ABX Diagnostics, Irvine, CA), whereas differential cell counts were performed manually using Dif-Quik-stained blood smears. Data were recorded according to the following categories: white blood cell (WBC); red blood cell (RBC); hemoglobin (HGB); hematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); red cell distribution width (RDW); packed cell volume (PCV); plasma protein (PP); segmented neutrophils (SEGS); band neutrophils (BANDS); lymphocytes (LYMPHS); monocytes (MONOS); eosinophils (EOS); basophils (BASOS); and reactive lymphocytes (RL). Biochemical tests were performed on coagulated blood using a Monarch Plus Analyzer (Instrumentation Laboratory, Lexington, MA). Data were recorded according to the following categories: albumin (ALB); alkaline phosphatase (ALP); alanine aminotransferase (ALT); total bilirubin (TBILI); blood urea nitrogen (BUN); creatinine (CR); calcium (CA); gamma-glutamyltransferase (GGT); glucose (GLU); total protein (TP); sodium (NA); potassium (K); chloride (CL); total carbon dioxide (TCO_2); and anion gap (AG). Phosphorus was recorded as (P) or phosphorus corrected (PCORR). Corrected phosphorus is a value that alleviates test interference caused by hemolysis, lipemia, or icterus. Urinalyses were performed using Chemstik 8 strips (Roche Diagnostics Corporation, Indianapolis, IN). Data were recorded according to the following categories: pH (UPH); protein (UP); glucose (UGLU); ketones (UKET); bilirubin (UBILI); urobilinogen (UROBIL); and blood (UBLD). Bumin protein (UBP) was determined by adding 5% sulfosalicylic acid-bumin reagent to urine and comparing to standards. With the exception of UPH, all of the above were characterized by the following: normal/negative; trace; 1+; 2+; or 3+. Specific gravity was determined via refractometer. The presence or absence of urine WBC or RBC was determined by direct microscopy of urine sediment. Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) status were recorded as positive or negative on the date of sample collection or if test results within the previous 3 months were available in the medical record.

Statistical Analysis

Cats were divided into 2 groups based on reciprocal antibody titers of 64 or greater to *B. henselae* or to *R. typhi* antigens (DEB-ELISA positive). A nested-study design was used in which the cats were grouped as either exposed or not exposed based on their antibody titers. A series of logistic regression models were used to assess the potential association of demographic factors, season of the year, and clinical and laboratory values with exposure to *B. henselae* or *R. typhi*. Continuous variables, such as weight, were coded into categories as noted above based on their previously recognized biologic validity.[23] Reference range laboratory values for cats, as derived by the clinical pathology laboratory at NCSU-CVM, were used as baseline values for comparison.

Variables were initially evaluated independently. Each variable was then added to a series of multivariable models to assess their potential association with having an antibody titer to *B. henselae* or *R. typhi* antigens. The independent univariate assess-

ment served as a baseline for the identification of confounders when compared with the multivariable models.[23,24] Changes to the maximum likelihood statistic (MLS), when assessed as variables, were added in a forward fashion to each model to improve validity or deleted to improve precision.[24] The numeric stability of the models were assessed by evaluating changes in the standard error of the maximum likelihood coefficient (MLC) (beta coefficient) as variables were added or deleted.[25] Odds ratios and corresponding 95% confidence intervals were derived from the MLC. Changes to the adjusted odds ratios and confidence intervals were sequentially evaluated to assess the potential association of the variable with having a titer and the variables contribution to the model. Confidence bounds that did not include unity (1.0) were considered significant. Odds ratios greater than 1.0 (unity) and corresponding confidence intervals greater than 1.0 were considered representative of the potential association of the variable with having a titer. Variables with odds ratios and confidence intervals less than 1.0 were considered potentially protective.

RESULTS

Serology

Of the 436 cat sera that were tested for reactivity to *B. henselae* and *R. typhi* antigens by IFA, 112 (26%) were reactive to *B. henselae* antigen, 93 (21%) were reactive to *R. typhi* antigen, and 31 (7%) were reactive to both test antigens. Of those sera that were reactive to *B. henselae* antigen (n = 112), reciprocal titers were 64 (n = 44), 128 (n = 30), 256 (n = 24), 512 (n = 11), and 1024 (n = 3). Of the serum samples that were reactive to *R. typhi* antigens (n=93), reciprocal titers were 64 (n = 60), 256 (n = 21), 512 (n = 8), 1024 (n = 3), and 2048 (n = 1). The *R. typhi* seroreactive samples with an IFA titer of 1:64 and higher were subjected to DEB-ELISA, where the initial testing dilution was 1:4. This indicates that antibodies in these samples were not blocked by the presence of the anti-*R. typhi* monoclonal antibody, and were therefore likely directed against non-*R. typhi* epitopes.

There did not appear to be cross reactivity between *B. henselae* and *R. typhi* antigens. Examination of 30 *R. typhi*-reactive samples showed no correlation with seroreactivity to *B. henselae*. In addition, the 3 cat sera with reciprocal *B. henselae* titers of 1024 were not reactive to *R. typhi* antigens, and 2 of 4 high-titer *R. typhi* sera (reciprocal titers 1024) did not react to *B. henselae* antigens. The other 2 *R. typhi* sera (reciprocal titers 1024 and 2048, respectively) were also reactive to *B. henselae* antigens at reciprocal titers of 128 and 256, respectively. Only 3 of 10 randomly selected *R. typhi* reactive sera were reactive to *R. rickettsii* antigens by IFA.

Demographics

Bartonella henselae seroreactive cats were more frequently intact males and were slightly younger than non-seroreactors (Table 1). There were no age, breed, or sex predilections among the *Rickettsia*-seroreactive cats. In addition, a majority of the cats included in this serosurvey were castrated or spayed (Table 1). A higher percentage of *B. henselae* seroreactors were DSH or DLH cats. Other breeds, including Siamese, Maine Coon, Persian, Himalayan, Russian Blue, Abyssinian, Somali, Balinese, Manx, Scottish Fold, Cymric, Birman, and Tonkinese, were more likely to be *B. henselae* non-seroreactors. A higher percentage of *B. henselae* non-seroreactors lived exclusively indoors (Table 1), whereas *B. henselae* seroreactors were more likely to have access to outdoor areas. In contrast, outdoor cats were less likely to be *R. typhi* seroreactive. Seroreactivity to *B. henselae* was found in a higher percentage (64.6%) of samples collected during the spring and summer (March-August) months; however, antibodies could be detected throughout the year. No seasonal trend in antibody prevalence was found for the *R. typhi* seroreactors. Cats that were seroreactive to *B. henselae* were more likely to have *R. typhi* antibodies.

Table 1. Univariate Assessment of the Association of Demographic, Clinical, and Laboratory Values With a Titer to *B. henselae* and *Rickettsia*.

		Bartonella henselae		*Rickettsia*	
Variable	**Group**	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**
Age (years)					
	1–5	1.0	—	1.0	—
	>5–10	0.96	0.55–1.68	0.69	0.39–1.21
	>10	0.94	0.54–1.65	0.63	0.35–1.13
Weight (kg)					
	<2.27	1.0	—	1.0	—
	2.28–4.55	2.07	0.44–9.34	1.69	0.36–7.84
	4.56–6.82	1.85	0.39–8.79	1.35	0.28–6.46
	>6.83	1.93	0.34–0.91	1.20	0.20–7.16
Sex					
	Female	1.0	—		
	Female spay	0.90	0.24–3.41		
	Male	2.5	0.37–18.0		
	Male castrate	1.4	0.37–5.28		
	Female	1.0	—	1.0	—
	Male	1.58	1.03–2.45	1.05	0.67–1.66
Breed					
	Other	1.0	—	1.0	—
	DLH	2.11	1.00–4.42	0.95	0.47–1.91
	DSH	2.15	1.12–4.13	0.91	0.50–1.64
Indoor/Outdoor					
	Indoor	1.0	—	1.0	—
	Outdoor	5.23	1.34–0.41	0.47	0.06–3.9
	Both	1.31	0.79–2.18	1.0	0.6–1.67
Month					
	Dec–Feb	1.0	—	1.0	—
	Mar–May	0.97	0.48–1.99	1.09	0.59–1.98
	June–Aug	1.67	0.91–3.06	0.87	0.43–1.77
	Sept–Nov	1.30	0.65–2.61	0.67	0.32–1.41
Temperature (˚F)					
	<100.4	1.24	0.72–2.12	1.13	0.64–1.99
	100.4–102.5	1.0	—	1.0	—
	>102.5	1.24	0.68–2.27	1.10	0.59–2.09
Pulse (beats per minute)					
	<160	1.16	0.68–1.98	0.73	0.39–1.38
	160–240	1.0	—	1.0	—
	>240	0.60	0.12–2.79	1.24	0.33–4.7
Respiration (breaths per minute)					
	<20	0.65	0.13–3.23	1.0	—
	20–30	1.0	—	1.00	0.19–5.07
	>30	0.95	0.54–1.67	1.41	0.74–2.68
Lymphadenopathy					
	No	1.0	—	1.0	—
	Yes	1.26	0.65–2.44	2.10	1.10–4.04

(continues on page 293)

		Bartonella henselae		*Rickettsia*	
Variable	**Group**	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**
Seizures					
	No	1.0	—	1.0	—
	Yes	1.47	0.54–4.02	1.92	0.70–5.26
Central nervous system dysfunction					
	No	1.0	—	1.0	—
	Yes	1.97	0.89–4.34	1.85	0.80–4.22
Interstitial nephritis					
	No	1.0	—	1.0	—
	Yes	0.96	0.09–9.36	0.93	0.1–8.42
Thromboembolism					
	No	1.0	—	1.0	—
	Yes	0.72	0.08–6.52	5.74	0.94–4.84
Hypertrophic cardiomyopathy					
	No	1.0	—	1.0	—
	Yes	1.80	0.99–3.26	1.12	0.57–2.17
Ocular abnormalities					
	No	1.0	—	1.0	—
	Yes	1.04	0.49–2.21	0.30	0.089–0.99
WBC ($\times 10^3/\mu L$)					
	<5.5	2.17	0.97–4.84	1.53	0.64–3.64
	5.5–19.5	1.0	—	1.0	—
	>19.5	1.43	0.84–2.43	1.15	0.66–2.01
RBC ($\times 10^6/\mu L$)					
	<5.0	1.08	0.50–2.32	0.77	0.32–1.81
	5.0–10.0	1.0	—	1.0	—
	>10.0	1.11	0.60–2.01	0.73	0.37–1.43
HGB (g/dL)					
	<8.0	1.15	0.58–2.28	0.67	0.29–1.55
	8.0–15.0	1.0	—	1.0	—
	>15.0	0.62	0.17–2.22	3.34	1.31–8.51
HCT (%)					
	<24.0	0.82	0.37–1.79	0.81	0.34–1.92
	24.0–45.0	1.0	—	1.0	—
	>45.0	0.86	0.48–1.54	1.41	0.80–2.50
MCV (fL)					
	<39.0	0.32	0.04–2.56	0.39	0.05–3.13
	39.0–55.0	1.0	—	1.0	—
	>55.0	0.96	0.30–3.05	0.59	0.13–2.67
MCH (pg)					
	<12.5	0.50	0.22–1.15	0.51	0.21–1.25
	12.5–17.5	1.0	—	1.0	—
	>17.5	1.19	0.30–4.69	0.85	0.18–4.10
MCHC (g/dL)					
	<31.0	0.97	0.61–1.57	0.58	0.36–0.94
	31.0–35.0	1.0	—	1.0	—
	>35.0	0.47	0.06–4.05	1.02	0.19–5.49

(continues on page 294)

		Bartonella henselae		*Rickettsia*	
Variable	**Group**	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**
PCV (%)					
	<23	1.04	0.52–2.11	1.0	—
	23–40	1.0	—	1.96	1.14–3.36
	>40	0.57	0.12–2.67		
PP (g/dL)					
	<6.0	0.72	0.20–2.62	0.26	0.19–0.35
	6.0–8.0	1.0	—	1.0	—
	>8.0	0.96	0.57–1.61	1.13	0.78–2.21
SEGS ($\times 10^3$)					
	<2.5	1.84	0.65–5.25	1.86	0.62–5.56
	2.5–12.5	1.0	—	1.0	—
	>12.5	1.10	0.67–1.80	1.4	0.85–2.34
BANDS ($\times 10^3$)					
	0–0.3	1.0	—	1.0	—
	>0.3	0.62	0.29–1.33	1.73	0.91–3.27
LYMPHS ($\times 10^3$)					
	<1.5	1.24	0.79–1.94	1.19	0.74–1.91
	1.5–7.0	1.0	—	1.0	—
	>7.0	4.10	1.18–3.69	1.31	0.34–5.01
MONOS ($\times 10^3$)					
	0–0.85	1.0	—	1.0	—
	>0.85	1.25	0.68–2.31	1.24	0.65–2.34
EOS ($\times 10^3$)					
	0–0.75	1.0	—	1.0	—
	>0.75	0.96	0.53–1.76	0.95	0.57–1.57
BASOS ($\times 10^3$)					
	0–0.1	1.0	—	1.0	—
	>0.1	1.48	0.86–2.52	1.08	0.60–1.93
RL ($\times 10^3$)					
	0–0.2	1.0	—	1.0	—
	>0.2	1.17	0.59–3.32	1.15	0.56–2.36
ALB (g/dL)					
	<2.7	1.0	—	1.0	—
	≥2.7	0.48	0.23–0.97	1.0	0.52–1.89
ALP (IU/L)					
	0–90	1.0	—	1.0	—
	>90	0.91	0.33–2.55	1.57	0.59–4.16
ALT (IU/L)					
	0–60	1.0	—	1.0	—
	>60	0.77	0.44–1.34	1.59	0.94–2.69
TBILI (mg/dL)	< 0.15	0.98	0.58–1.65	1.21	0.74–1.99
	0.15 – 0.20	1.0	—	1.0	—
	> 0.20	1.39	0.82–2.34	2.26	0.86–5.95
BUN (mg/dL)					
	<14	1.38	0.51–3.70	2.15	0.83–5.60
	14–38	1.0	—	1.0	—
	>38	1.54	0.88–2.68	1.10	0.58–2.10

(continues on page 295)

		Bartonella henselae		*Rickettsia*	
Variable	**Group**	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**
CR (mg/dL)					
	<0.8	0.98	0.19–4.93		
	<1.8			1.0	—
	0.8–1.8	1.0	—		
	>1.8	0.98	0.59–1.63	0.66	0.37–1.18
CA (mg/dL)					
	<9.2	1.22	0.78–1.90	0.89	0.55–1.42
	9.2–10.2	1.0	—	1.0	—
	>10.2	1.89	0.75–4.77	0.35	0.10–1.20
GGT (IU/L)					
	0–5.0	1.0	—	1.0	—
	>5.0	1.17	0.22–6.14	3.12	0.82–1.86
GLU (mg/dL)					
	<150	1.0	—	1.0	—
	≥150			1.56	0.94–2.61
TP (g/dL)					
	<6.3	0.78	0.43–1.40	0.64	0.33–1.24
	6.3–8.7	1.0	—	1.0	—
	>8.7	1.92	0.66–5.54	1.80	0.60–5.43
NA (mmol/L)					
	<146	2.51	1.10–5.74	1.81	0.75–4.36
	146–156	1.0	—	1.0	—
	>156	1.18	0.64–2.17	0.87	0.44–1.72
K (mmol/L)					
	<4.0	1.17	1.07–2.74	1.05	0.46–1.98
	4.0–4.8	1.0	—	1.0	—
	>4.8	1.10	0.55–2.22	0.96	0.46–1.98
CL (mmol/L)					
	<115	1.50	0.80–2.82	1.29	0.66–2.55
	115–130	1.0	—	1.0	—
	>130	0.24	0.06–1.05	0.49	0.14–1.68
TCO_2 (mmol/L)					
	<16	1.15	0.63–2.07	1.16	0.62–2.19
	16–26	1.0	—	1.0	—
	>26	1.00	0.20–5.02	1.29	0.26–6.54
Albumin/globulin ratio					
	<5			0.75	0.09–6.48
	5–15	1.0	—	1.0	—
	>15			1.56	0.87–2.78
PCORR (mg/dL)					
	<3.1	1.27	0.48–3.40	0.43	0.10–1.88
	3.1–7.1	1.0	—	1.0	—
	>7.1	1.10	0.45–2.68	1.60	0.54–4.70
P (mg/dL)					
	<3.1			0.73	0.39–1.38
	3.1–7.1	1.0	—	1.0	—
	>7.1			1.24	0.33–4.70

(continues on page 296)

		Bartonella henselae		*Rickettsia*	
Variable	**Group**	**Odds Ratio**	**95% Confidence Interval**	**Odds Ratio**	**95% Confidence Interval**
Specific gravity					
	<1.030	1.0	—	1.0	—
	≥1.030	1.55	0.91–2.65	1.33	0.74–2.39
UPH					
	<6.0	1.35	0.73–2.50	0.98	0.51–1.91
	6.0–7.0	1.0	—	1.0	—
	>7.0	1.12	0.47–2.66	0.83	0.32–2.14
UP					
	None	1.0	—	1.0	—
	Yes	1.53	0.50–4.66	6.02	0.80–5.56
UGLU					
	None	1.0	—	1.0	—
	Yes	0.79	0.36–1.74	1.73	0.85–3.54
UKET					
	None	1.0	—	1.0	—
	Yes	6.64	0.59–74.31	4.03	0.56–2.22
UBILI					
	None	1.0	—	1.0	—
	Yes	0.78	0.39–1.57	0.97	0.48–1.95
UROBIL					
	None	Not enough elevated data to run			
	Yes				
UBLD					
	None	1.0	—	1.0	—
	Yes	1.43	0.81–2.50	1.38	0.77–2.49
UBP					
	None	1.0	—	1.0	—
	Yes	1.18	0.64–2.17	1.06	0.57–1.98
Urine WBC (/hpf)					
	0–5.0	1.0	—	1.0	—
	>5.0			1.16	0.62–2.19
Urine RBC (/hpf)					
	None	1.0	—	1.0	—
	Yes	1.26	0.69–2.28	1.61	0.84–3.11
FELV					
	Negative	1.0	—	1.0	—
	Positive	1.75	0.68–4.46	0.91	0.29–2.84
FIV					
	Negative	1.0	—	1.0	—
	Positive	1.86	0.30–11.44	0.89	0.10–8.19
T4					
	1–4	1.0	—	1.0	—
	>4	2.02	0.53–7.69	2.95	0.77–11.40

Clinicopathological Findings

Lymphadenopathy, a history of seizures or other central nervous system abnormalities, and cardiomyopathy (but not thromboembolism) were associated with *B. henselae* seroreactivity by univariate analysis. Similar

associations were found in the *Rickettsia* seroreactors, with the exception that thromboembolism (odds ratio 5.7) rather than cardiomyopathy was found in seroreactors. Lymphocytic plasmacytic hepatitis, granulomatous hepatitis, granulomatous splenitis, and lymphocytic nephritis were not associated with *B. henselae* or *R. typhi* seroreactivity.

Mean values for temperature, pulse rate, and respiratory rate were similar, and within reference ranges for *B. henselae* seroreactors and non-seroreactors. *Rickettsia typhi* seroreactors were more likely to have a low body temperature (below 100.4˚F [38.3˚C]). Mean CBC values and serum biochemical results were similar for both *B. henselae*- and *R. typhi*-positive and negative cats (Table 1). *Bartonella henselae* seroreactors were more likely to be leukopenic due to a decrease in neutrophils, and at times, lymphocytes. *Rickettsia typhi* seroreactors were more likely to have polycythemia (elevated HCT and HGB concentration). In regard to biochemical parameters, *B. henselae* seroreactivity was associated with a tendency for hyperbilirubinemia, increased serum urea nitrogen concentrations (without an accompanying increase in serum CR concentration), increased serum protein, increased CA concentration with a low serum P concentration, and a tendency for decreased NA, K, CL, and bicarbonate. Presence of *R. typhi* antibodies was associated with an increase in GGT above 5.0 IU/L. No variables detected by urinalyses were associated with *B. henselae* or *R. typhi* antibodies. Urine tended to be concentrated, and UP, UKET, UBLD, WBC, and RBC were found in the urine of a majority of the cats tested; however, sediment changes were found in a higher percentage of *B. henselae* seroreactors. In the majority of the cats tested, UGLU, UBILI, and UROBIL were also present in the urine, however, a higher percentage of these substances was found in the urine of *B. henselae* non-seroreactors. Mean values for UPH and specific gravity were similar and within reference ranges for both *B. henselae* and *R. typhi* seroreactors and non-seroreactors. In this study, infections with FeLV were found more often than FIV infection, and a higher percentage of *B. henselae* seroreactors were infected with FeLV or FIV than were *B. henselae* non-seroreactors. Mean values for T4 were similar and within reference range for both *B. henselae* and *R. typhi* seroreactors.

Independent assessment of each demographic, clinical, and laboratory variable suggested the potential association of numerous study factors with detection of *B. henselae* or *Rickettsia* antibodies (Tables 1 and 2). A series of multivariable logistic regression models were evaluated using the independent assessment as a reference for variable selection and recognition of confounding factors.

In the final logistic model developed, cats with *B. henselae* titers were more likely to be DSH and to reside exclusively outdoors. Cats with *B. henselae* titers were also more likely to have blood in their urine (Table 2). In the final model, cats with a lymphadenopathy (Table 3) and elevated PCV were more likely to have a titer suggesting exposure to *R. typhi*. The wide confidence intervals for the variable PCV reflect some missing values for the RBC data. Cats with ocular disorders were less likely to have a titer, suggesting prior exposure to *R. typhi*.

DISCUSSION

We simultaneously examined both *B. henselae* and *Rickettsia* antibody prevalence in a cat population from North Carolina. A combination of IFA testing and DEB-ELISA was used to refine the source of seroreactivity to *R. typhi* antigens in cats. However, lack of specific *R. typhi* antibodies in 9 cat sera tested positive by IFA but negative by DEB-ELISA clearly indicated presence of rickettsial antibodies that were not directed toward SPA of *R. typhi*. Based on clinical experience, North Carolina cats are rarely infested with ticks, but frequently infested with fleas. The positive DEB-ELISA test results might reflect exposure to other flea

Table 2. Final Logistic Regression Model for the Potential Association of Demographic, Clinical, and Laboratory Values With the Presence of a Titer to *B. henselae*.

Variable	Group	Adjusted Odds Ratio	95% Confidence Interval
Breed			
	DSH	2.09	1.07–4.066
	Other	1.0	—
Housing			
	Outdoor exclusive	5.19	1.28–21.06
	Indoor/outdoor, indoor exclusive	1.0	—
Urine blood			
	Blood present in urine	2.31	1.18–4.55
	No blood in urine		
Alternative Presentation			
Breed			
	DSH	2.15	1.08–4.27
	Other	1.0	—
Housing			
	Outdoor exclusive	6.08	1.23–29.98
	Indoor/outdoor, indoor exclusive	1.0	—
Urine blood			
	Blood present in urine	2.55	1.27–5.13
	No blood in urine	1.0	—
Lymphocyte count			
	Lymphocytosis	3.55	0.70–17.87
	Normal/lymphopenia	1.0	—
More Detailed Presentation			
Breed			
	DSH	1.69	1.04–2.56
	Other	1.0	—
Housing			
	Outdoor exclusive	5.60	1.34–23.35
	Both	1.20	0.64–2.25
	Indoor	1.0	—
Urine blood			
	Blood present in urine	2.31	1.17–4.55
	No blood in urine	1.0	—
With Lymphocyte Count			
Breed			
	DSH	1.69	1.07–2.67
	Other	1.0	—
Housing			
	Outdoor exclusive	6.40	1.26–32.58
	Both	1.20	0.64–2.26
	Indoor	1.0	—
Urine blood			
	Blood present in urine	2.39	1.21–4.74
	No blood in urine	1.0	—
Lymphocyte count			
	Lymphocytosis	1.41	0.81–2.44
	Within normal limits/ normal/lymphopenia	1.0	—

Table 3. Final Logistic Regression Model for the Potential Association of Demographic, Clinical, and Laboratory Values With the Presence of Rickettsial Non–*R. typhi* Antibodies.

Variable	Group	Adjusted Odds Ratio	95% Confidence Interval
Lymphadenopathy			
	Yes	2.38	1.21–4.69
	No	1.0	—
PCV	≥45	10.08	2.57–39.54
	<45	1.0	—
RBC	<5.0	0.64	0.27–1.54
	5.0–10.00	1.0	—
	>10.0	0.40	0.16–0.99
Ocular abnormalities	Yes	0.20	0.05–0.88
	No	1.0	—
Alternative Presentation			
Lymphadenopathy	Yes	2.28	1.17–4.46
	No	1.0	—
PCV	≥45	10.02	2.56–39.19
	<45	1.0	—
RBC	<10.0	1.0	—
	≥10.0	0.42	0.17–1.03
Ocular abnormalities	Yes	0.21	0.05–0.91
	No	1.0	—

transmitted *Rickettsia*, such as *R. felis*, rather than tick-transmitted *R. rickettsii*. In fact, we detected low reactivity to *R. rickettsii* antigen in only 3 out of 10 *R. typhi* cat sera. Recent genetic and antigenic analysis clearly placed *R. felis* among spotted-fever group rickettsia.[16] However, human sera with reactivity to *R. felis* have higher cross-reactive antibody titer to *R. typhi* antigen compared with *R. rickettsii* antigens.[11,12] In future studies, serological testing in cats should be complemented by molecular detection techniques and/or attempts at rickettsial isolation. Unfortunately, *R. felis* is an extremely difficult organism to isolate from a human patient or animal sample, the organism is difficult to maintain in the laboratory setting, and molecular detection in blood samples may be insensitive due to very low quantities of template DNA.[14-16] *Rickettsia felis* has been successfully isolated and cultured from fleas, but not from sick animals or people, suggesting that isolation from fleas collected from domestic indoor/outdoor cats may be required to further define the epidemiology of *Rickettsia* antibodies in cats.[26] Although both *B. henselae* and *R. felis* were discovered during the past decade, there is currently more clinical and epidemiological information regarding *B. henselae* than *R. felis* in cats, humans, or other animal species.

In recent years, numerous studies have documented a high prevalence of *B. henselae* antibodies in cats residing in warmer climates (geographic locations that are conducive to high levels of flea infestation, which results in frequent transmission of *B. henselae* among cat populations).[1] Flea transmission of *B. henselae* to SPF cats has been demonstrated experimentally and, although yet to be completely elucidated, the flea appears to act as both a mechanical

and biological vector.[4] Once infected with *B. henselae*, cats can remain persistently infected with a relapsing pattern of bacteremia for months to years.[8,17,20] Cats experimentally infected with *B. henselae* by blood transfusion or by inoculation of culture-grown *B. henselae* have developed clinical abnormalities including fever, transient anemia, eosinophilia, reproductive and behavioral abnormalities, and neurological dysfunction.[8,17-20] Based upon the predilection of *B. henselae* to invade erythrocytes and vascular endothelial cells in cats, pathological consequences would seem likely. However, whether, or to what extent, chronic infections with *B. henselae* cause disease manifestations or pathology in cats is yet to be clearly established. Due to the high level of bacteremia detected in cats residing in flea-endemic areas, such as the southeastern United States, establishing a cause-and-effect relationship will be difficult, particularly if the bacteria can contribute to slow, insidious damage to tissues over a protracted period of time (months to years).[1,27] Disease associations might be more readily established in geographic areas such as Norway or the United Kingdom, where *B. henselae* seroprevalences are low.[28,29]

Two studies have provided epidemiological evidence that *B. henselae* seroreactivity correlates with disease manifestations in immunocompetent and immunocompromised cats, respectively.[29,30] A study from Switzerland, a country with a relatively low *B. henselae* seroprevalence, indicated that fever, lymphadenopathy, and renal disease were correlated with *B. henselae* antibodies.[29] A study from Japan, involving retroviral-infected cats, found a significant correlation between *B. henselae* antibodies and stomatitis, gingivitis, and lymphadenopathy in cats that were co-infected with FIV and *B. henselae* compared with FIV-infected cats that lacked detectable *B. henselae* antibodies.[26] An association of *B. henselae* seroreactivity with stomatitis and lymphadenopathy has also been proposed for cats from North America.

In this study, cats that were *B. henselae* seroreactive were more likely to be male DLH or DSH cats that were allowed access to outdoor areas as compared to purebred cats that were maintained exclusively indoors. This tendency may be related to a greater likelihood of flea infestation in cats that are allowed to roam or have access to a flea-infested outdoor environment. There is increasing evidence to support a sylvatic cycle of flea infestation due to the infestation of wild mammals, particularly raccoons and opossums, with cat fleas.[3] Unlike some previous studies, age was not associated with a decreasing frequency of *B. henselae* antibodies.[17] Although *B. henselae* antibodies were not a risk factor for lymphadenopathy in this study, lymphocytosis was associated with *B. henselae* antibodies. This particular association is perhaps even more important than the odds ratio (6.61) supports, in as much as our study population predominantly consisted of severely sick cats referred to a tertiary care center. Therefore, a stress-induced decrease in lymphocyte numbers would be anticipated in this cat population. As is typical of other chronic infections, for example canine ehrlichiosis or leishmaniasis, lymphocytosis might be expected with chronic *Bartonella* infections in cats, but unlike ehrlichiosis or leishmaniasis in dogs, *B. henselae* infection in cats does not appear to consistently induce hyperglobulinemia. Based on this study, detection of lymphocytosis accompanied by hematuria in a male DSH cat that resides in an outdoor environment would be consistent with *B. henselae* infection.

In contrast, seroreactivity, presumably associated with non–*R. typhi*-derived rickettsial antibodies, was found in cats with lymphadenopathy and needs to be considered in the design of future studies. As lymphadenopathy has been reported in 2 studies involving cats with *B. henselae* seroreactivity, it is possible that both organisms may contribute to lymph node enlargement in cats. Of unknown clinical significance, cats with *R. typhi* antibodies were more likely to have a reduced body temperature and an elevated HCT and HGB concentration. As

these findings would be consistent with decreased cardiac output, potentially due to feline hypertrophic or restrictive cardiomyopathy, an association with cardiomyopathy seems unlikely as the heart rate in the *R. typhi*-seroreactive cats was not increased.

The Veterinary Teaching Hospital at NCSU-CVM is primarily a referral-based teaching hospital. Therefore, the cats included in this study may not be representative of the likelihood of exposure to *B. henselae* or *Rickettsia* observed in cats seen by practitioners or in other parts of the country. Exposure to both organisms appears to be frequent in our cat population. The multivariable models provide a reasonable representation of exposure history to *B. henselae* and *R. typhi* in cats seen in a referral population of sick cats at our institution. External validation at other institutions and in other geographic areas is needed to further define the risk factors that contribute to the exposure and the extent to which infection with *B. henselae*, *R. typhi*, and other *Rickettsia*, such as *R. felis*, contribute to clinicopathological abnormalities in cats.

ACKNOWLEDGMENTS

We wish to thank David Walker, University of Texas Medical Branch at Galveston, for the gift of the *R. typhi* SPA monoclonal antibody and Kester Crosse from Abdu Azad's Laboratory, University of Maryland, School of Medicine, Baltimore, MD, who assisted with the *R. typhi* IFA as a summer research student.

REFERENCES

1. Breitschwerdt EB, Kordick DL: *Bartonella* infection in animals: Carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clin Microbiol Rev* 2000;13:428–438.
2. Karem KL, Paddock CD, Regnery RL: *Bartonella henselae*, *B. quintana*, and *B. bacilliformis*: Historical pathogens of emerging significance. *Microbes Infect* 2000;2:1193–1205.
3. Azad AF, Radulovic S, Higgins JA, Noden BH, Troyer JM: Flea-borne rickettsioses: Ecologic considerations. *Emerg Infect Dis* 1997;3:319–327.
4. Finkelstein JL, Brown TP, O'Reilly KL, Wedincamp JJ, Foil LD: Studies on the growth of *Bartonella henselae* in the cat flea (Siphonaptera: Pulicidae). *J Med Entomol* 2002;39:915–919.
5. Radulovic S, Higgins JA, Jaworski DC, Dasch GA, Azad, AF: Isolation, cultivation, and partial characterization of the ELB agent associated with cat fleas. *Infect Immun* 1995;63:4826–4829.
6. Higgins JA, Radulovic S, Schriefer ME, Azad AF: *Rickettsia felis*: A new species of pathogenic rickettsia isolated from cat fleas. *J Clin Microbiol* 1996;34:671–674.
7. Chomel B, Kasten RW, Floyd-Hawkins KA, et al: Experimental transmission of *Bartonella henselae* by the cat flea. *J Clin Microbiol* 1996;34:1952–1956.
8. Kordick DL, Breitschwerdt EB: Relapsing bacteremia after blood transmission of *Bartonella henselae* to cats. *Am J Vet Res* 1997;58:492–497.
9. Jacobs RF, Schutze GE: *Bartonella henselae* as a cause of prolonged fever and fever of unknown origin in children. *Clin Infect Dis* 1998;26:80-84.
10. Schriefer ME, Sacci JB, Dumler JS, Bullen MG, Azad AF: Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. *J Clin Microbiol* 1994;32:949–954.
11. Koehler JE, Sanchez MA, Garrido CS, et al: Molecular epidemiology of bartonella infections in patients with bacillary angiomatosis-peliosis. *N Engl J Med* 1997;337:1916–1917.
12. Zavala-Velazquez JE, Ruiz-Sosa JA, Sanchez-Elias RA, Becerra-Carmona G, Walker DH: *Rickettsia felis* rickettsiosis in Yucatan. *Lancet* 2000;356:1079–1080.
13. Raoult D, LaScola B, Enea M, et al: A flea associated rickettsia pathogenic for humans. *Emerg Infect Dis* 2001;7:73–81.
14. Richter J, Fournier PE, Petridou J, Haussinger D, Raoult D: *Rickettsia felis* infection acquired in Europe and documented by polymerase chain reaction. *Emerg Infect Dis* 2002;8:207–208.
15. Oliveira RP, Galvao MAM, Mafra CL, et al: *Rickettsia felis* in *Ctenocephalides* spp. fleas, Brazil. *Emerg Infect Dis* 2002;8:317–319.
16. Rolain JM, Franc M, Davoust B, Raoult D: Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis* and *Wolbachia pipientis* in cat fleas, France. *Emerg Infect Dis* 2003;9:338–342.
17. Radulovic S, Speed R, Feng HM, Taylor C, Walker DH: EIA with species-specific monoclonal antibodies: a novel seroepidemiological tool for determination of the etiological agent of spotted fever rickettsiosis. *J Infect Dis* 1993;138:1292–1295.
18. Vaden SL, Levine J, Breitschwerdt EB: A retrospective case-control of acute renal failure in 99 dogs. *J Vet Intern Med* 1997;11:58–64.

19. Hosmwer DW, Lemeshow S: *Applied Logistic Regression*. John Wiley and Sons, Inc.: Canada; 1997:8–18, 25–76, 88–175.

20. Schlesseman JJ: *Case-control Studies*. Oxford University Press: New York; 1992:254–259.

21. 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;172:1145–1149.

22. Bergh K, Bevanger L, Hanssen I, Loseth K: Low prevalence of *Bartonella henselae* infections in Norwegian domestic and feral cats. *APMIS* 2002;110:309–314.

23. Birtles RJ, Laycock G, Kenny MJ, Shaw SE, Day MJ: Prevalence of *Bartonella* species causing bacteremia in domesticated and companion animals in the United Kingdom. *Vet Rec* 2002;151:225–229.

24. Abbott RC, Chomel BB, Kasten RW, et al: Experimental and natural infection with *Bartonella henselae* in domestic cats. *Comp Immunol Microbiol Infect Dis* 1997;20:41–51.

25. Guptill 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.

26. Guptill L, Slater LN, Wu CC, et al: Evidence of reproductive failure and lack of perinatal transmission of *Bartonella henselae* in experimentally infected cats. *Vet Immunol Immunopathol* 1998;65:177–189.

27. 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;37:1536–1547.

28. Glaus T, Hofmann-Lehmann R, Greene C, Glaus B, Wolfensberger C, Lutz H: Seroprevalence of *Bartonella henselae* infection and correlation with disease status in cats in Switzerland. *J Clin Microbiol* 1997;35:2883–2885.

29. Ueno H, Hohdatsu T, Muramatsu Y, Koyama H, Morita C: Does coinfection of *Bartonella henselae* and FIV induce clinical disorders in cats? *Microbiol Immunol* 1996;40:617–620.

30. La Scola B, Meconi S, Fenollar F, Rolain JM, Roux V, Raoult D: Emended description of *Rickettsia felis* (Bouyer et al. 2001), a temperature-dependent cultured bacterium. *Int J Syst Evol Microbiol* 2002;52:2035–2041.