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Prevalence, Risk Factors, and Genetic Diversity of *Bartonella henselae* Infections in Pet Cats in Four Regions of the United States

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Blood was collected from a convenience sample of 271 pet cats aged 3 months to 2 years (mean age, 8 months, median and mode, 6 months) between May 1997 and September 1998 in four areas of the United States (southern California, Florida, metropolitan Chicago, and metropolitan Washington, D.C.). Sixty-five (24%) cats had *Bartonella henselae* bacteremia, and 138 (51%) cats were seropositive for *B. henselae*. Regional prevalences for bacteremia and seropositivity were highest in Florida (33% and 67%, respectively) and California (28% and 62%, respectively) and lowest in the Washington, D.C. (12% and 28%, respectively) and Chicago (6% and 12%, respectively) areas. No cats bacteremic with *B. clarridgeiae* were found. The 16S rRNA type was determined for 49 *B. henselae* isolates. Fourteen of 49 cats (28.6%) were infected with 16S rRNA type I, 32 (65.3%) with 16S rRNA type II, and three (6.1%) were coinfected with 16S rRNA types I and II. Flea infestation was a significant risk factor for *B. henselae* bacteremia (odds ratio = 2.82, 95% confidence interval, 1.1 to 7.3). Cats ≥13 months old were significantly less likely to be bacteremic than cats ≤6 months old (odds ratio = 0.18, 95% confidence interval, 0.05 to 0.61). Flea infestation, adoption from a shelter or as a stray cat, hunting, and being from Florida or California were significant risk factors for *B. henselae* seropositivity. DNA fingerprint was significantly associated with region ($P = 0.03$) and indoor/outdoor status of cats ($P = 0.03$).

Bartonella henselae causes cat scratch disease, endocarditis, parenchymal bacillary peliosis, neuroretinitis, bacillary angiomatosis, central nervous system disorders, and prolonged or relapsing fever with bacteremia in human beings. Many patients affected by the last three syndromes are immunocompromised, but *B. henselae* also causes similar disease syndromes in immunocompetent individuals (10, 20, 21, 24, 25, 30, 33). Another *Bartonella* species, *B. clarridgeiae*, has been associated with a cat scratch disease-like syndrome (17).

Domestic cats are considered reservoirs and vectors for human infections with *B. henselae* (5, 15, 31, 36) and for *B. clarridgeiae*. Cat scratches and ownership of young cats are the strongest risk factors for zoonotic cat scratch disease and bacillary angiomatosis (31, 36). Transmission of *B. henselae* among cats is thought to occur primarily through fleas (6).

Feline *B. henselae* isolates are genetically diverse (2, 11, 16, 19, 22, 26–29). Two different *B. henselae* types based on partial 16S rRNA gene sequences have been described (3), with multiple DNA fingerprint patterns identified for each type (29). Infection with one 16S rRNA type of *B. henselae* appears to protect from reinfection with homologous strains of *B. henselae*, but not in all cases against heterologous challenge (34, 35). The results of these studies suggest that vaccines developed for use in cats to protect against *B. henselae* infection must include antigenic material from at least both 16S rRNA types. Whether infection with individual DNA fingerprint types of *B. henselae* protects cats from infection with any other DNA fingerprint types has not been investigated. A study of cats living in and around Paris, France, showed that of 57 cats infected with *B. henselae*, 41 were infected with rRNA type II, 14 were infected with rRNA type I, and 2 were coinfected with rRNA types I and II. In addition, eight cats were infected with *B. clarridgeiae* and *B. henselae* and 15 with *B. clarridgeiae* only (9).

Current recommendations for preventing transmission of *B. henselae* from cats to human beings include avoidance of cat scratches or cat bites and maintaining pet cats free of fleas. Results of a recent epidemiologic study of *Bartonella* in cats in France suggested that cats that had been owned for >6 months or were from single-cat households were less likely to be infected with *Bartonella* (9).

The prevalence of bacteremia among young pet cats, the most likely vectors for zoonotic *B. henselae* infections, has not been systematically evaluated. Most previous studies of prevalence of *B. henselae* bacteremia in cats in the United States have used convenience samples of cats of various ages in shelters, at veterinary clinics, and cats belonging to veterinary students. These studies have not evaluated the genetic diversity of *B. henselae* isolates (5, 15; D. L. Kordick, K. H. Wilson, B. C. Hegarty, H. A. Berkhoff, and E. B. Breitschwerdt, Intersci. Conf. Antimicrob. Agents Chemother., p. 199, 1994). A nationwide survey of cat sera submitted for a variety of tests at commercial laboratories in the United States found that seroprevalence of cats seropositive for *B. henselae* varied by geographic region and was highest in areas with warm or humid climates that were well suited to support flea populations (13).

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The goals of the study reported here were to determine the proportion of young pet cats receiving veterinary care in four areas of the United States that are bacteremic and/or serologically positive for *B. henselae*, use DNA fingerprinting and 16S rRNA type to evaluate the genetic diversity of *B. henselae* isolates obtained from young pet cats, and characterize risk factors for *B. henselae* bacteremia or seropositivity in young pet cats.

(This work was presented in part at the European Working Group on *Rickettsia*, *Coxiella*, *Ehrlichia* and *Bartonella*–American Society for Rickettsiology Joint Meeting, June 1999, and in part at the 14th Sesquiannual Meeting of the American Society for Rickettsiology, September 2000.)

MATERIALS AND METHODS

Animals. Pet cats ≤2 years of age seen at private veterinary hospitals between May 1997 and September 1998 for routine veterinary care in four geographic regions of the United States (southern California, Florida, the Washington, D.C., area, and the Chicago, Ill., area) were evaluated. Questionnaires completed by owners and veterinarians provided demographic information and health histories of the cats. Information collected for risk factor analysis included date (season) of blood collection, geographic region, age, breed, gender and neutering status, source of the cat, feline leukemia virus and feline immunodeficiency virus test results; whether the cats had ever had fleas, lived in single-cat or multicat homes, used a litter box, had been ill or treated with antibiotics within 2 weeks of blood collection, were indoor or outdoor cats, hunted, or had been bitten by another animal. Owners were given a description of the study and asked for informed consent before blood was collected from their cats.

Bacteriology. Blood was collected for bacterial culture with lysis centrifugation tubes (Isolator) and for serology with serum separator tubes (Becton Dickinson, Franklin Lakes, N.J.). Veterinarians centrifuged serum separator tubes before shipment. Blood samples were shipped overnight to the Animal Disease Diagnostic Laboratory at Purdue University. Blood from lysis centrifugation tubes was immediately plated on fresh chocolate agar for quantitative culture as previously described (7, 30). The identity of *B. henselae* colonies was verified by colony morphology and Gram staining characteristics, indole and catalase tests, and labeling with polyclonal goat anti-*B. henselae* serum and fluorescein isothiocyanate-labeled rabbit anti-goat immunoglobulin G (IgG) as previously described (7). Isolates were subcultured and frozen at −70°C in trypticase soy broth with 20% glycerol. Sera were aliquoted and frozen at −70°C for enzyme immunoassay for anti-*B. henselae* IgM and IgG and anti-*B. clarridgeiae* IgM and IgG antibodies.

Serology. Enzyme immunoassays were performed as previously described (8), and the mean optical density (OD) of triplicate determinations was calculated. An OD index was constructed by dividing the mean OD measurement for each cat by the mean OD + 2 standard deviations of a group of specific-pathogen-free cats that were blood culture negative. An OD index of greater than 1.00 was considered significant for exposure to *Bartonella*.

If the veterinarian submitting the blood samples had not tested a cat for feline leukemia virus or feline immunodeficiency virus, then serum was tested with an enzyme-linked immunosorbent assay test kit for feline leukemia virus antigen and anti-feline immunodeficiency virus IgG antibodies (Snap Combo Test; IDEXX, Portland, Maine).

DNA extraction and PCR analysis from cultured bacteria. Enterobacterial repetitive intergenic consensus sequence PCR was used for DNA fingerprinting with primers ERIC1R and ERIC2R (32). *Bartonella henselae* isolates were grown on fresh chocolate agar and collected into saline. Cells were lysed with a buffer of 50 mM Tris, 25 mM EDTA, 0.5% Tween 20, and 0.5% Triton X-100. DNA was isolated with a standard phenol-chloroform-isoamyl alcohol extraction and ethanol precipitation (14). Each 25-μl reaction mixture included 200 ng of DNA, 4 mM $MgCl_2$, 400 μM each deoxynucleoside triphosphate, 2 μM each primer, 2 U of *Taq* polymerase (Promega, Madison, Wis.), 1× *Taq* buffer (Promega), and 10% dimethyl sulfoxide. The amplification reaction included denaturation at 95°C for 7 min, then 30 cycles of denaturation at 90°C for 30 s, annealing at 48°C for 60 s and extension at 65°C for 60 s, then a final extension step at 65°C for 8 min. Products were separated on 2% agarose gels, stained with ethidium bromide, and photographed. DNA fingerprint patterns were evaluated by visual inspection independently by three investigators (C.-C.W., L.G., H.H.). Patterns were considered different if they differed by at least one distinct band based on size. Intensity differences between bands were not considered differences.

16S rRNA type by PCR. *Bartonella* 16S rRNA type was determined by PCR with previously described primers 16SF and either BH1 or BH2 (3). DNA was isolated with proteinase K (14). Reactions were conducted in a 50 μl volume and included 5 μl of DNA extract, 20 pmol of each primer, and 200 μM each deoxynucleoside triphosphate in 50 mM KCl–10 mM Tris-HCl–0.1% Triton X-100–3.5 mM $MgCl_2$–0.2 U of *Taq* DNA polymerase (Promega) under the following conditions: 3 min of denaturing at 95 C, then 30 cycles of denaturing for 20 s at 95°C, annealing at 56°C for 30 s, and extension at 72°C for 1 min, then a final extension step at 72°C for 5 min. Products were separated by electrophoresis in a 2% agarose gel and visualized with ethidium bromide. Negative controls included tubes containing a reaction mixture without a DNA template and tubes containing a reaction mixture plus extraction blanks (sterile saline processed exactly as described above for bacterial isolates in a proportion of approximately one negative control to 10 isolates).

Statistical analysis. Statistical analyses were performed with SAS version 8.2 software (SAS software for version 8; SAS Institute Inc., Cary, N.C., 1999). Logistic regression (29a) was used to obtain odds ratios and 95% confidence limits for potential risk factors for *B. henselae* bacteremia in cats.

The following risk factors were evaluated individually by logistic regression models to determine if they were associated with *B. henselae*-positive culture: age in months, gender, neutering status, source of cat (friend/self/breeder, stray/shelter, or pet store/newspaper), fleas (never versus ever), region (Washington, D.C., Chicago, Florida, or southern California), season, antibiotic use in the past 2 weeks (yes versus no), breed (purebred versus mixed breed), cats in household (single cat versus multiple cats), indoor/outdoor (indoor versus indoor/outdoor), hunting (yes versus no), ever sick (yes versus no), litter box use (never, occasional, or regular), and ever bitten by another animal (yes versus no). Variables that were statistically significant in univariate analyses at $P < 0.1$ were entered into a multivariable logistic regression model. This process was repeated to identify potential risk factors for a cat having positive anti-*B. henselae* IgG serology. A P value of <0.05 was considered significant.

Chi square and Fisher's exact tests were used to assess univariate associations between DNA fingerprint pattern and risk factors. A chi square test for linear trend was used to assess the relationships between cat age and bacteremia or seropositivity. Results were considered significant at $P < 0.05$.

TABLE 1. *B. henselae* culture and serology results for 271 pet cats

Region	No. of cats	No. (%) culture positive	No. (%) serum IgG positive
California	97	27 (28)	60 (62)
Florida	94	31 (33)	63 (67)
Chicago	48	3 (6)	6 (12)
Washington, D.C.	32	4 (12)	9 (28)

RESULTS

Animals. Blood was collected from 271 cats; 97 (35.8%) from southern California, 94 (34.7%) from Florida, 31 (11.8%) from the Washington, D.C., area, and 48 (17.7%) from the Chicago, Ill., area. Fifty-two percent of the cats were male and 48% were female; 61% of the cats were neutered. The median age of the 261 cats for which age was reported was 6 months (range, 3 to 26 months) and the average age was 8 months. There were 13 (5%) purebred cats; the remaining cats were of mixed breeding. Of 238 cats for which source was known, 105 (44%) had been adopted as strays or from a shelter, 96 (40%) were bred at home or by a breeder, and 36 (16%) were obtained through newspaper advertisements or from a pet store. All of the cats were negative for feline leukemia virus and feline immunodeficiency virus.

Bacteriology and serology. Sixty-five cats (24%; 95% confidence interval, 19 to 29%) had *B. henselae* bacteremia (Tables 1 and 2). Seven blood samples grew contaminants and were not

TABLE 2. Univariate analysis of risk factors for *B. henselae* bacteremia or seropositivity[a]

Risk factor	No. of tested samples	Bacteremia		No. of tested samples	Seropositivity	
		OR	95% CL		OR	95% CL
Age (mo)						
0–6	150	1.00		156	1.00	
7–12	73	0.814	0.43, 1.54	73	1.68	0.96, 2.96
13–18	28	0.299	0.09, 1.04	28	0.62	0.27, 1.42
19+	13	0.207	0.03, 1.64	14	2.77	0.83, 9.21
Neutering status						
Intact male	36	1.00		39	1.00	
Neutered male	97	2.27	0.79, 6.46	99	1.59	0.75, 3.37
Intact female	64	1.73	0.57, 5.29	64	1.44	0.64, 3.21
Neutered female	64	2.82	0.95, 8.32	66	1.95	0.87, 4.36
Source						
Friend, self, or breeder	96	1.00		98	1.00	
Stray or shelter	105	2.22	1.14, 4.32	110	2.06	1.18, 3.59
Store or newspaper	37	1.28	0.50, 3.29	37	0.42	0.18, 0.96
Flea infestation						
Never	98	1.00		99	1.00	
Ever	162	3.84	1.89, 7.80	168	3.84	1.89, 7.8
Region						
Washington, D.C.	29	1.00		32	1.00	
Chicago area	47	0.43	0.09, 2.06	48	0.36	0.11, 1.15
Florida	93	3.12	1, 9.77	94	5.19	2.14, 12.55
Southern California	95	2.48	0.79, 7.80	97	4.14	1.73, 9.91
Season						
Winter	105	1.00		106	1.00	
Spring	43	0.69	0.30, 1.57	44	1.84	0.88, 3.86
Summer	31	0.44	0.15, 1.24	31	0.41	0.18, 0.95
Autumn	85	0.62	0.31, 1.19	90	0.72	0.41, 1.26
Antibiotics use in past 2 wks						
No	220	1.00		225	1.00	
Yes	25	1.82	0.76, 4.35	27	0.66	0.29, 1.48
Hunting						
No	198	1.00		205	1.00	
Yes	21	2.84	1.14, 7.10	21	3.89	1.27, 11.97
Breed						
Purebred	13	1.00		13	1.00	
Mixed breed	249	1.84	0.4, 8.56	256	2.61	0.78, 8.71
Multiple-cat home						
No	76	1.00		77	1.00	
Yes	150	0.983	0.53, 1.84	156	0.71	0.41, 1.23
Ever sick						
No	175	1.00		181	1.00	
Yes	81	1.36	0.75, 2.48	82	0.95	0.56, 1.59
Litter box use						
Never	11	1.00		11	1.00	
Occasionally	12	0.875	0.16, 4.87	12	0.07	0.01, 0.75
Regularly	196	0.583	0.16, 2.08	203	0.12	0.01, 0.92
Ever bitten						
No	204	1.00		210	1.00	
Yes	28	1.03	0.41, 2.56	29	1.1	0.5, 2.4
Environment						
Indoor	177	1.00		182	1.00	
Outdoor only or indoor and outdoor	50	1.02	0.52, 1.98	52	1.19	0.67, 2.13

[a] Cats with contaminated cultures were not included in the analysis. OR, odds ratio; 95% CL, 95% confidence limits.

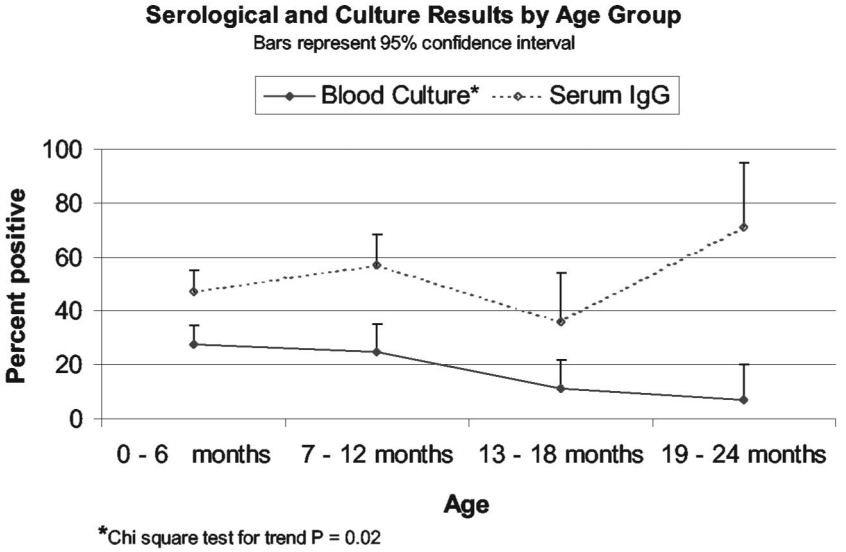

FIG. 1. Proportions of cats that were blood culture positive and seropositive by age. Seven cats with contaminated blood cultures were not included in the data presented in this graph. The proportion of cats with *B. henselae* bacteremia decreased significantly as cat age increased ($\chi^2 = 5.361$, $P = 0.02$). The proportion of cats seropositive for *B. henselae* increased with increasing age, but this trend was not statistically significant ($\chi^2 = 0.7533$, $P = 0.3854$).

considered positive or negative. Fifty-seven of the 65 bacteremic cats (88%; 95% confidence interval, 80 to 96%) were serologically positive. Fifty-six (86%) were IgG seropositive for *B. henselae* and 1 cat was IgM but not IgG seropositive for *B. henselae*. A total of 11 bacteremic cats (17%; 95% confidence interval, 8 to 26%) were IgM seropositive for *B. henselae*. Seventy-eight (39%; 95% confidence interval, 32 to 46%) of 199 nonbacteremic cats were seropositive (IgM or IgG) and 121 (61%; 95% confidence interval, 54 to 68%) were seronegative. Six nonbacteremic cats (3%) were IgM seropositive for *B. henselae*; 2 of these cats were negative for anti-*B. henselae* IgG serum antibodies. Eight (12%; 95% confidence interval, 4 to 20%) bacteremic cats were seronegative. The positive and negative predictive values of serum IgG as an indicator of *B. henselae* bacteremia were 43% and 93%, respectively.

No cultures yielded *B. clarridgeiae*. However, 15% of *B. henselae* bacteremic cats and 3% of nonbacteremic cats were IgG seropositive for *B. clarridgeiae*. The proportion of *B. clarridgeiae* seropositive cats in the Washington, D.C., area was 9% (95% confidence interval, −1 to 19%), in the Chicago, Ill., area it was 10% (95% confidence interval, 1.5 to 18.5%), and in both Florida and southern California it was 5% (95% confidence interval, 1 to 10%). In contrast, the proportion of cats seropositive for *B. henselae* in southern California was 62% (95% confidence interval, 52 to 72%) and in Florida it was 67% (95% confidence interval, 58 to 76%), whereas the proportion of cats in the Washington, D.C., area seropositive for *B. henselae* was 28% (95% confidence interval, 12 to 44%) and in the Chicago area it was 12% (95% confidence interval, 3 to 21%).

Univariate risk factor analysis. The chi square test for trend showed that the proportion of cats with *B. henselae* bacteremia decreased significantly as cat age increased ($\chi^2 = 5.361$, $P = 0.02$). The proportion of cats seropositive for *B. henselae* increased with increasing age, but this trend was not statistically significant ($\chi^2 = 0.7533$, $P = 0.3854$, Fig. 1). Only one of 11 cats aged 3 months was bacteremic, and only three of the 3-month-old cats had anti-*Bartonella* serum antibodies. No cats <3 months of age were evaluated. Forty-eight (74%) of 65 bacteremic cats were ≤6 months old, while 119 (60%) of 199 culture-negative cats were ≤6 months old.

Six risk factors were significantly associated with bacteremia or seropositivity at $P < 0.1$ (Table 2) and were included in the multivariate model. Risk factors associated with bacteremia included flea exposure ($P = 0.002$), gender ($P = 0.06$), source of cat ($P = 0.02$), hunting ($P = 0.03$), and region ($P = 0.05$). Risk factors associated with seropositivity included flea exposure ($P = 0.0002$), gender ($P = 0.1$), source ($P = 0.01$), never used a litter box ($P = 0.04$), hunting ($P = 0.02$), and region ($P = 0.001$).

Multivariate risk factor analysis. Cats that had fleas at any time in their life had a significantly increased risk of being blood culture positive for *B. henselae* (odds ratio = 2.82, 95% confidence interval, 1.10 to 7.27; Table 3), but were not significantly more likely to be IgG seropositive compared to cats that never had fleas (odds ratio = 1.74, 95% confidence interval, 0.83 to 3.64). Cats >13 months of age had a significantly decreased risk of being blood culture positive for *B. henselae* (odds ratio = 0.18; 95% confidence interval, 0.05 to 0.61) compared to cats aged 0 to 6 months. Cats obtained from a shelter or as stray cats were significantly more likely to be seropositive (odds ratio = 2.02, 95% confidence interval, 1.03 to 3.99) than cats bred at home or by a breeder, but not significantly more likely to be bacteremic (odds ratio = 2.00, 95% confidence interval, 0.94 to 4.22).

Cats from Florida or California were significantly more likely to be IgG seropositive (Florida odds ratio = 4.21, 95% confidence interval, 1.41 to 12.51; California odds ratio = 4.26, 95% confidence interval, 1.4 to 13) than cats from the Washington, D.C., area, but were not significantly more likely to be bacteremic (Florida odds ratio = 2.11, 95% confidence interval, 0.5 to 8.6; California odds ratio 3.2, 95% confidence interval, 0.8 to 13.3). Cats that hunted were significantly more likely

TABLE 3. Risk factors for *B. henselae* bacteremia or seropositivity: multivariate analysis[a]

Risk factor	No. of samples tested	Bacteremia		No. of samples tested	Seropositivity	
		OR	95% CL		OR	95% CL
Age (mo)						
0–6	150	1.00		156	1.00	
7–12	73	0.54	0.24, 1.26	73	1.58	0.73, 3.42
13+	41	0.18	0.05, 0.61	42	0.90	0.32, 2.51
Gender						
Male	133	1.00		133	1.00	
Female	128	1.41	0.69, 2.88	128	1.42	0.73, 2.75
Neutering status						
Neutered	161	1.00		165	1.00	
Intact	100	0.47	0.22, 1.02	103	0.70	0.35, 1.39
Source						
Friend, self, or breeder	96	1.00		98	1.00	
Stray or shelter	105	2.00	0.94, 4.22	110	2.02	1.03, 3.99
Store or newspaper	37	2.08	0.62, 6.99	37	1.03	0.35, 3.07
Fleas						
Never	98	1.00		99	1.00	
Ever	162	2.82	1.10, 7.27	168	1.74	0.83, 3.64
Region						
Washington, D.C.	29	1.00		32	1.00	
Chicago	47	0.84	0.21, 6.05	48	0.57	0.14, 2.35
Florida	93	2.11	0.52, 8.58	94	4.21	1.41, 12.51
California	95	3.20	0.77, 13.32	97	4.26	1.38, 13.15
Hunting						
No	198	1.00		205	1.00	
Yes	21	3.00	0.77, 13.32	21	5.71	1.12, 29.01

[a] Variables selected for this model were significant at $P < 0.10$ in a univariate analysis. Data are missing for 52 cats. Cats with contaminated cultures were not included in the analysis. OR, odds ratio; 95% CL, 95% confidence limits.

to be seropositive (odds ratio = 5.71, 95% confidence interval, 1 to 29) than were cats that never hunted, but were not significantly more likely to be bacteremic (odds ratio = 3.00, 95% confidence interval, 0.96 to 9.26).

DNA fingerprinting. Fifty (77%) of the 65 *B. henselae* isolates were evaluated by DNA fingerprinting; the remaining isolates did not regrow for DNA isolation. Isolates evaluated included two from the Chicago area, four isolates from the Washington, D.C., area, 25 isolates from Florida, and 19 isolates from southern California. The DNA fingerprint patterns of these cultures were classified into five categories (Ia, Ib, IIa, IIb, and IIc). Categories Ia and Ib differed by the presence or absence of one band at approximately 270 bp. All isolates in categories IIa, IIb, and IIc lacked a band at 680 bp that was present in all isolates in groups Ia and Ib. In addition, isolates in category IIb lacked a band at approximately 580 bp and the single isolate in category IIc lacked bands at approximately 580, 550, and 320 bp and had a distinct band at approximately 560 bp. The Houston-1 isolate (ATCC 49882) was classified in category IIa.

For statistical analysis, the isolates in categories Ia and Ib were grouped (group I), and isolates in categories IIa, IIb, and IIc were grouped (group II) (Fig. 2). Statistical analyses for association of DNA fingerprint pattern with geographic region were done only with data from southern California and Florida because of the small number of isolates from the Chicago and Washington, D.C., areas. Three of the four fingerprinted isolates from the Washington, D.C., area were in group II and one was in group I. Both isolates from the Chicago area that were fingerprinted were in group II.

DNA fingerprint group I was most likely to be isolated in Florida (14 [78%] of 18 group I isolates) while group II was most likely to be isolated in California (15 [58%] of 26 group II isolates, $P = 0.03$). DNA fingerprint pattern was also significantly associated with the indoor/outdoor status of the cat ($P = 0.03$). Eighty-eight percent of evaluated isolates with DNA fingerprint pattern II (23 of 26 isolates) and 56% (10 of 18 isolates) with DNA fingerprint pattern I were from indoor cats. Seventy-four percent of 97 cats tested in California were indoor cats, compared with 67% of 94 cats tested in Florida.

No other risk factors (age, breed, gender, neuter status, number of cats in the household, presence or absence of fleas, season of the year, litter box use, hunting, being bitten by other animals, history of antibiotic treatment, or previous illness) were significantly associated with DNA fingerprint pattern.

16S rRNA type. The 16S rRNA type was determined one year following DNA fingerprinting for 49 (75%) of the culture positive cats (Table 4); isolates from the remaining cats could not be regrown for DNA isolation. Cats for which 16S rRNA type was determined included two from the Chicago area, four from the Washington, D.C., area, 22 from Florida, and 21 from southern California. Three cats (6%, 95% confidence interval, −1 to 13%) were coinfected with 16S rRNA types I and II. Of cats infected with a single rRNA type of *B. henselae*, 14 cats (30%; 95% confidence interval, 17 to 43%) were infected with 16S rRNA type I, and 32 (70%; 95% confidence interval, 53 to 83%) cats were infected with 16S rRNA type II. The majority of isolates from cats in southern California were 16S rRNA type II (16 of 21; 76%; 95% confidence interval, 58 to 94%), while equal numbers of isolates from cats in Florida were 16S rRNA type I or II (10 each). All isolates tested from cats in the Chicago and Washington, D.C., areas were 16S rRNA type II. For the 47 isolates from all regions analyzed by both 16S rRNA and DNA fingerprinting methods, most isolates that were 16S rRNA type I were in DNA fingerprint group I (13 of 16; 81%; 95% confidence interval, 62 to 100%) and most isolates that were 16S rRNA type II were in DNA fingerprint group II (24 of 31; 77%; 95% confidence interval, 62 to 92%).

Eight (58%) of 14 cats infected with *B. henselae* rRNA type I were strictly indoor cats, versus 22 (69%) of 32 cats infected with *B. henselae* rRNA type II ($P = 0.51$).

DISCUSSION

Sixty-five (24%) of 271 young pet cats had *B. henselae* bacteremia, and 138 (51%) of the cats were serologically positive for *B. henselae*. The regional prevalences of seropositivity were similar to those reported previously (13), though generally higher. Information is not available regarding the age and health status of the cats in the previous study, and the serologic methods were different (enzyme immunoassay versus indirect fluorescent antibody tests), and therefore direct comparisons cannot be made.

The negative predictive value of IgG serology for bacteremia was high (93%), but the positive predictive value of IgG serology for bacteremia was low (42%). These findings are similar to those in previous studies with indirect fluorescent antibody

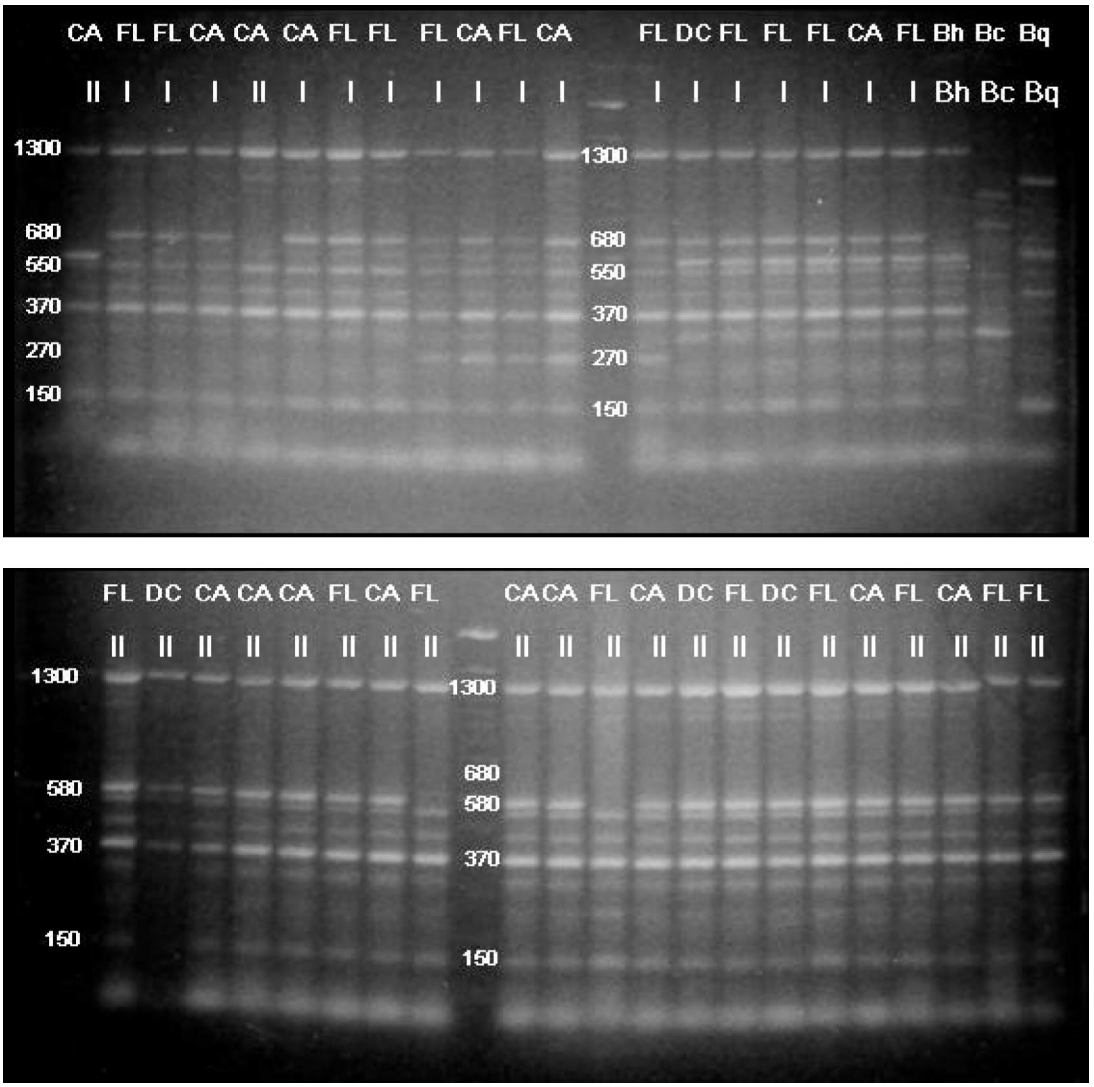

FIG. 2. DNA fingerprint patterns of cats, identified by region and fingerprint pattern. The top row of labeling above each lane indicates the region (FL, Florida; CA, southern California; D.C., Washington, D.C., area), and the second row indicates DNA fingerprint pattern (I, pattern I; II, pattern II). Bh, DNA fingerprint of *Bartonella henselae* (ATCC strain 49882); Bc, DNA fingerprint of *Bartonella clarridgeiae* (strain from Leonard Slater); and BQ, DNA fingerprint pattern of *Bartonella quintana* (strain from Leonard Slater). Lanes 13 in the upper gel and 9 in the lower gel contain DNA molecular size markers; sizes are shown to the left in base pairs.

tests (5, 9), and enzyme immunoassay tests (2) and suggest that serologic testing may have some utility to rule out bacteremia in evaluating cats being considered as prospective pets for immunocompromised owners in that cats negative for IgG serum antibodies to *B. henselae* have a low probability of being infected. The low number of cats seropositive for IgM anti-*B. henselae* antibodies (11 [17%] of 65 bacteremic cats) suggests that IgM enzyme immunoassay serology is not a sensitive test for screening or diagnosis.

Although no *B. clarridgeiae* was cultured from the blood of any cats in this study, *B. clarridgeiae* was cultured from 10 to 21% of blood samples from cats in other studies (9, 11, 17, 18). The culture method employed in the current study may have decreased the probability of isolating *B. clarridgeiae* (B. Chomel, personal communication) in that the blood was not collected into EDTA and frozen before plating, and no media

TABLE 4. Distribution of DNA fingerprint patterns and 16S rRNA types by region[a]

Region	No. of cats				
	DNA fingerprint group I	DNA fingerprint group II	16S rRNA type I	16S rRNA type II	16S rRNA types I & II
California	4	15	4	16	1
Florida	14	11	10	10	2
Washington, D.C.	1	3	0	4	0
Chicago	0	2	0	2	0

[a] For two cats from California, 16S rRNA type was determined but DNA fingerprint was not; for one cat from California and two cats from Florida, DNA fingerprint was determined but 16S rRNA type was not. For the other cats represented in the table, both DNA fingerprint type and 16S rRNA type were determined.

other than chocolate agar were used. The regional difference in the distribution of cats seropositive for *B. henselae* and *B. clarridgeiae* has not been reported previously.

The decreasing proportion of cats with bacteremia and increasing proportion of cats seropositive for *B. henselae* with increasing cat age, as well as the positive association of bacteremia with a history of flea infestation, were expected and in agreement with the findings of a previous study (5). Flea infestation was previously significantly associated with *B. henselae* seropositivity, but not with bacteremia (9), whereas in the study reported here, a history of flea infestation was positively associated with bacteremia but not a positive serologic status. The population of cats tested in the previous study was somewhat older (age range, 3 months to 17 years, mean age, 2.2 years; mode, 8.4 months) whereas all of the cats in the current study were ≤2 years of age (mean age, 8 months; mode 6, months). Older cats are less likely to be bacteremic and more likely to be seropositive than younger cats (5). Therefore, flea-infested cats in the previous study may have been less likely to be bacteremic versus seropositive as a function of their age. Another possible explanation is that antibody concentration decreased enough following exposure so that at the time of sampling, enzyme immunoassay results were negative in some cats in the current study that had previously been positive. However, antibody levels have been shown to persist longer than detectable bacteremia (7, 23) in some cats.

Cats acquired as strays or from a shelter were significantly more likely than cats bred at home or acquired from friends or a breeder to be bacteremic. This is in agreement with findings of a study of French cats (9). Cats that hunted were significantly more likely to be seropositive than cats that did not hunt. This is the first report of an association between hunting and *Bartonella* and suggests that there may be sources for *Bartonella* infection for cats other than fleas. It is also possible that hunting is a surrogate marker for outdoor cats and/or flea exposure. However, there was not a significant association between indoor/outdoor status of cats and either bacteremia or positive serologic status.

The lack of a significant association of bacteremia or seropositivity with season of the year was not expected, given the association with flea infestation and the reported seasonality of cat scratch disease in people (12, 21). The flea population density may not change enough with season to affect transmission of *Bartonella* among cats in Florida and southern California, the regions where the majority of cats resided. In addition, the seasonality of cat scratch disease in human beings may be more related to the time of year when most people are exposed to young cats rather than to other factors.

In contrast to the findings of a recent French study (9) that reported that cats from multiple cat households were significantly more likely to be bacteremic or seropositive than cats in single cat households, the results of the study reported here show no significant association between number of cats in a household and the likelihood a cat was seropositive or bacteremic.

The regional distribution of DNA fingerprint patterns supports the hypothesis that genetic variants of *B. henselae* develop over time as the organism is passed among cats in the same area. Whether infections with these genetic variants provide heterologous cross protection against new infections, or whether largely homologous cross protection occurs as was demonstrated for different 16S rRNA types in other studies (34, 35) has not been investigated. In addition, it is not known whether all DNA types of *B. henselae* cause disease in human beings (1, 3, 28). Investigators (3, 4, 28) have demonstrated *B. henselae* of different 16S rRNA types or DNA patterns in clinical samples from infected human beings.

The results reported here indicate that healthy young pet cats are at risk for infection with *B. henselae*. Although there were few 3-month-old cats evaluated, the facts that only 1 of 11 3-month-old cats was bacteremic and only 3 3-month-old cats had anti-*B. henselae* serum antibodies suggest that vaccination of cats ≤3 months old may be an effective means of preventing infection of cats and therefore transmission of *B. henselae* infection to human beings. Vaccines developed to prevent infection of cats with *B. henselae* should protect against all of the common genetic variants of *B. henselae*.

The results of this study also indicate that cats with a background of being stray animals or living in a shelter, cats with flea infestations, and cats that hunt are at the greatest risk for *B. henselae* infection. Cats from private homes that are free of fleas and that are seronegative for *B. henselae* are the least likely to have *B. henselae* bacteremia.

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