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BARTONELLA HENSELAE ANTIBODY PREVALENCE IN FREE-RANGING AND CAPTIVE WILD FELIDS FROM CALIFORNIA

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ABSTRACT: In order to determine the importance of wild felids in the epidemiology of *Bartonella* spp. infection, 136 Nobuto strips or serum samples from free-ranging mountain lions (*Felis concolor*) and bobcats (*Felis rufus*) captured in California (USA) between 1985 and 1996 were tested for *B. henselae* antibodies (titer ≥ 1:64) using an immunofluorescence test. Similarly, 124 serum samples from 114 captive wild cats representing 26 species or subspecies collected between 1991 and 1995 were retrieved from the serum banks of four California zoological parks. Fifty-three percent (33/62) of the bobcats, 35% (26/74) of the mountain lions, and 30% (34/114) of the captive wild felids (genera *Acinonyx*, *Panthera* and *Felis*) had *B. henselae* antibodies. In captive wild felids, prevalence varied widely among the species, but seropositivity was more likely to occur in the genus *Felis* than in the genus *Acinonyx* or *Panthera*. Prevalence was evenly distributed between sexes, except for free-ranging mountain lions. Antibody prevalence ranged from 25% in 0- to 2-yr-old captive felids to 35% in cats ≥9-yr-old, but the highest antibody titers were observed in cats <5-yr-old.

Key words: Bartonellosis, *Bartonella henselae*, bobcat, *Felis concolor*, *Felis rufus*, mountain lion, serologic survey, wild cats, zoological park.

INTRODUCTION

Cat scratch disease (CSD) was initially described by Debré et al. (1950), yet the causative agent of CSD remained obscure until 1992 when *Bartonella henselae* was implicated by serologic and microbiologic studies (Regnery et al., 1992a; Dolan et al., 1993). Certainty of a bacterial origin issued from the works of Wear et al. (1983), who observed bacilli in lymph nodes and in cutaneous early lesions of CSD patients. Cat scratch disease in humans is typically a benign, subacute regional lymphadenopathy resulting from dermal inoculation of *B. henselae*, a bacterium that had been initially isolated from patients with bacillary angiomatosis (Regnery et al., 1995; Schwartzman, 1996). Bacillary angiomatosis (BA) is a vascular proliferative disease mainly seen in human immunodeficiency virus (HIV)-infected persons. Several other clinical manifestations have been reported, such as bacteremia, peliosis hepatis, endocarditis, neuroretinitis, and aseptic meningitis. Within the framework of serologic studies directed to determine the presence of antibodies against the newly discovered agent of BA, Regnery et al. (1992c) and Zangwill et al. (1993) reported frequent occurrence (84 to 88%) of antibodies against *B. henselae* in CSD patients, leading to the implication of *B. henselae* as the agent of CSD.

Exposure to cats was one of the several criteria used in the diagnosis of CSD, but the exact role of the cat as a vector or reservoir of the infectious agent causing CSD was not clear (Kirkpatrick and Whiteley, 1987; Margileth, 1993). The initial isolation of *B. henselae* from the blood of a healthy serologically positive cat (Regnery et al., 1992b) and later from a high per-

centage of naturally infected cats (Koehler et al., 1994; Chomel et al., 1995), along with the demonstration that cats remain bacteremic for months (Kordick et al., 1995; Abbott et al., 1997) suggest that cats play a major role as reservoir for the bacterium.

Various serosurveys in domestic cat populations have been conducted in the United States using an immunofluorescent antibody test. A serological and epidemiological study of banked cat sera collected between 1980 and 1985 in Baltimore (Maryland, USA) by Childs et al. (1994) indicated a *Bartonella* spp. antibody prevalence of 15% (87/592). In another survey, Childs et al. (1995) reported a prevalence of 28% (370/1,314) from cats in various parts of the United States. In a serosurvey of 518 sick cats from North Carolina (USA), 109 (21%) cats were positive to *B. henselae* (Breitschwerdt and Kordick, 1995). A sero-epidemiologic survey, incorporating 628 samples from throughout North America, identified an overall prevalence of 28% (175/628), ranging from a low of 4 to 7% in the Midwest and Great Plains region to 60% in the Southeast (Jameson et al., 1995). High seroprevalence appeared to correlate with warm, humid climates. These warm, humid areas with the highest seroprevalence also were the ones reported to have the highest number of potential arthropod vectors, including fleas. The experimental demonstration of domestic cat infection by *B. henselae* from infected cat fleas, *Ctenocephalides felis* (Chomel et al., 1996), the high percentage of *B. henselae* infected fleas collected on cats in a heavily *B. henselae* infected cat colony, and the demonstration that cat fleas can harbor and shed *B. henselae* in their feces (Higgins et al., 1996) strongly support this epidemiological association.

There are an estimated 5,100 mountain lions (*Felis concolor*) (Paul-Murphy et al., 1994) and an equal or larger number of bobcats (*Felis rufus*) (P. Swift, unpubl. data) in California (USA). Possible interaction with free-roaming domestic cats could occur in the wild or accidentally in zoological parks causing potential exchange of ectoparasites between wild felids and domestic cats. It is also possible that wild felids could be naturally infected with *Bartonella* spp. and serve as reservoirs of these bacteria. Therefore, it was of interest to determine the prevalence of *B. henselae* antibodies in free-ranging wild cats captured or killed in California and in captive wild felids kept in certain zoological parks. We also examined the association between seropositivity with genus, species, age, sex, and geographical origin for free-ranging felids.

MATERIAL AND METHODS

Serum samples or Nobuto filter strips (Advantec/MSS Inc., Pleasanton, California, USA) from 74 free-ranging mountain lions and 62 bobcats were obtained from the California Department of Fish and Game (Rancho Cordova, California, USA) and the California Department of Health Services, Vector Borne Disease Section (Sacramento, California, USA). Samples were collected from 1985 through 1996 and kept frozen at −20 C until tested. There were 21 Nobuto strips and 41 serum samples for the bobcats, and 25 Nobuto strips and 49 serum samples for the mountain lions. County of origin, age and sex were available for most of these animals. Most (92%) of the bobcats tested were adults (57/62), and 33 (53%) were males. The bobcats were trapped in seven different California counties: Alpine, Kern, Marin, Mendocino, Plumas, Santa Clara and Siskiyou (Fig. 1). The majority (81%, 50/62) of the bobcats were captured in the northern coastal range (37°N to 40°N, 121°30′W to 124°W), the remaining animals were from southern California (34°50′N to 35°45′N, 117°30′W to 120°W; Kern County: nine animals), and the Sierra Nevada (38°N to 40°N, 119°40′W to 123°30′W; three animals). Mountain lions were captured in 20 California counties (38°30′N to 42°N; 114°W to 124°22′W). County of capture was not available for one mountain lion. Mountain lions were from five counties in southern California (Kern, San Bernardino, San Diego, Los Angeles and Ventura), five coastal counties (San Luis Obispo, Monterey, Sonoma, Mendocino and Humboldt), and ten counties in the Sierra Nevada or its foothills (Amador, Butte, El Dorado, Inyo, Lassen, Placer, Plumas, Sacramento, Sierra and Siskiyou) (Fig. 1). Seventy percent (52/74) of the moun-

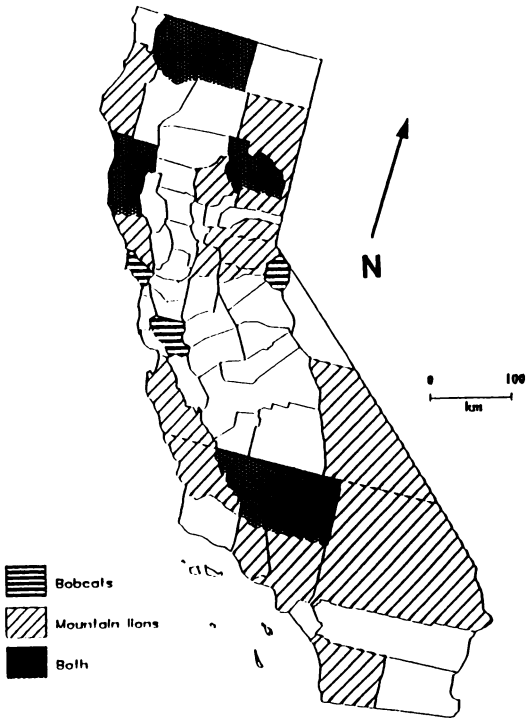

FIGURE 1. Counties of origin of free-ranging bobcats and mountain lions tested for *Bartonella henselae* antibodies in California (USA).

tain lions were males and 73% (54/74) were adults (≥2-yr-old). Data concerning sex, estimated age, date and county where the animals were captured were collected.

One hundred and twenty-four serum samples of captive wild cats were retrieved from the serum banks of the San Diego Zoo (San Diego, California, USA), the Sacramento Zoo (Sacramento, California, USA), the Micke Grove Zoo (Lodi, California, USA) and the Folsom Zoo (Folsom, California, USA). Ninety sera were collected from 81 wild felids housed at the San Diego Zoo between 1991 and 1995. Twenty species or subspecies of cats were represented. A single serum sample was available from 74 animals, while two or three samples were available from seven animals [two cheetah (*Acinonyx jubatus*), one Persian leopard (*Panthera pardus*), one Indochinese tiger (*Panthera tigris*), two clouded leopards (*Neofelis nebulosa*), and one fishing cat (*Felis viverinus*)]. An additional serum sample was collected from a domestic cat (*Felis catus*) that was freely roaming on the Zoo grounds. Medical records and computerized animal data were searched to ascertain the age and sex of the animal. Twenty-one serum samples from 20 animals (two samples from one cheetah) representing 13 species or subspecies of wild cats were collected between March 1990 and September 1994 from animals at the Sacramento Zoo. Data concerning age and sex were available for most of the animals. Single serum samples were collected between June 1992 and July 1994 from 10 wild felids, representing five species or subspecies of wild cats, housed at the Micke Grove Zoo. Single serum samples were collected between January and May 1994 from three mountain lions housed at the Folsom Zoo. Age and sex were available for most of these animals.

Antibody titers against *B. henselae* were determined using an indirect immunofluorescence antibody (IFA) test (Regnery et al., 1992c). For antigen preparation, *B. henselae*, originally isolated from a naturally infected cat, was cultivated with *Felis catus* whole fetus (Fcwf) cells in tissue culture media for 3 to 5 days. Infected Fcwf cells were applied to each well of multi-well, super-cured, heavy teflon coated slides (CelLine Associates, Inc., Neufield, New Jersey, USA) and incubated for 24 hr to allow the cells to adhere to the slides. Slides were then rinsed in phosphate-buffered saline (PBS, pH 7.4, SIGMA, St. Louis, Missouri, USA), air dried, acetone fixed, and stored at −20 C after air-drying a final time. Cat sera were diluted at 1:64 in PBS containing 5% skim milk and added to the test wells of the slides, and incubated for 35 min at 37 C. After washing in PBS, fluorescein labeled goat anti-cat IgG (whole-molecule immunoglobulin G; Cappel, Organon Teknika Corp., Durham, North Carolina, USA) was used as the conjugate. Slides were incubated for 20 min at 37 C. Positive and negative controls were included on each test slide. Intensity of fluorescence of the bacteria was graded subjectively on a scale of 1 to 4. Fluorescence equal to or greater than a value of two at a dilution of 1:64 was considered to be a positive result (Childs et al., 1994). Positive sera were two-fold serially diluted to obtain an endpoint titer. Blind reading in duplicate of the slides was performed for all the serum samples by the same readers.

Nobuto filter strips, initially dipped in the animal blood and air dried, were cut thin into two equal pieces into a 1.5 ml Eppendorf vial and 0.4 ml of borate buffer (1.5 M NaCl, 0.5 M H_3BO_3, 1 M NaOH), pH 8.0 added. The vial was kept overnight at 4 C and then heat inactivated for 20 min at 60 C. The paper strips were then removed after being pressed with a plastic rod and two drops of sheep red blood cells added. The vials were left overnight at 4 C. The vials were then centrifuged for 5 min at 2,000 rpm. Samples were kept frozen at −20 C until tested. By comparison with known pos-

itive serum samples it was determined that a dilution of 1:8 of the Nobuto extract was equivalent to a 1:64 dilution of a serum sample.

Descriptive data were analyzed using Epi Info version 6.03 (Dean et al., 1994). Frequency distributions were obtained and chi-square tests for homogeneity for 2 × 2 contingency tables were used to examine the statistical significance of any associations. Fisher's exact test was used to examine antibody prevalence differences between the San Diego Zoo and the Sacramento Zoo.

RESULTS

Among the 62 bobcats tested, 33 (53%) were seropositive for *B. henselae*. The prevalence was lower in mountain lions; 26 (35%) of 74 mountain lions were seropositive. Prevalence was not statistically different between adult and juvenile bobcats and mountain lions. In mountain lions, 40% (8/20) of the juveniles (<2-yr-old) were seropositive and 33% (18/54) of the adults had *B. henselae* antibodies. For the mountain lions with a given age estimate, 50% (7/14) of the lions <2-yr-old and 39% (11/28) of the lions ≥2-yr-old were seropositive. There was no major difference in prevalence by sex in bobcats [19 of 33 (57%) males and 14 of 29 (48%) females], but male mountain lions [22 of 51 (43%) males] were more likely than females [4 of 22 (18%) females] to be seropositive [relative risk (RR) = 2.37, 95% confidence interval (CI) = 0.93, 6.08]. Antibody prevalence varied widely among the geographic locations where the wild felids were captured (Table 1).

Antibodies to *Bartonella henselae* were found in 34/114 (30%) animals from four different California zoos. Prevalence ranged from 0% at Folsom Zoo where only three young mountain lions were tested to 28% (23 of 81 cats) at the San Diego Zoo, 30% (3/10) at Micke Grove Zoo and 40% (8 of 20 animals) at the Sacramento Zoo.

Antibody prevalence varied greatly among the different species of wild cats (Table 2). The seroprevalence was 18% (2/11) in the cheetah, 17% (9/54) among cats of the genus *Panthera,* which include lions (*P. leo*), tigers (*P. tigris*), leopards (*P. pardus*) and jaguars (*P. onca*), and 47% (23/49) among cats belonging to the genus *Felis*. Therefore, the cats of the genus *Felis* were almost three times more likely to be seropositive for *B. henselae* than animals belonging to the genera *Panthera* and *Acinonyx* (RR = 2.77; 95% CI = 1.50, 5.13). Small cats (average adult weight <20 kg) were 2.5 times more likely than the large cats (average adult weight ≥ 20 kg) to be seropositive (RR = 2.53; 95% CI = 1.46, 4.41). Antibody prevalence was twice as high in cheetahs and cats of the genera *Panthera* at the Sacramento Zoo (2/7) than the same species at the San Diego Zoo (7/49), but the difference was not statistically significant (Fisher's exact test, $P = 0.3$). Seropositivity was similar for the cats belonging to the genus *Felis* between the Sacramento Zoo (6/13; 46%) and the San Diego Zoo (16/32; 50%).

There was no difference in antibody prevalence by sex; 30% of the males (17/56) and 28% of the females (16/58) were seropositive. Age was available for 108 animals (95%). Antibody prevalence fluctuated between age groups, but increased from 25% in the 0- to 2-yr-old group to 35% in cats ≥9-yr-old (Table 3). However, within the 0- to 2-yr-old group, seroprevalence was the lowest (12%) in the cats <1-yr-old (3/25) and the highest (37%) in

TABLE 1. Prevalence of *Bartonella henselae* antibodies in free-ranging bobcats and mountain lions in California.

Geographic region	Bobcats prevalence[a] (%)	Mountain lions prevalence (%)
Mendocino County	0/7 (0)	
Marin County	18/24 (75)	
Santa Clara County	10/19 (53)	
Humboldt, Sonoma, Mendocino Counties		7/22 (32)
Monterey, San Luis Obispo Counties		2/10 (20)
Southern California	5/9 (55)	3/10 (30)
Sierra Nevada	0/3 (0)	13/31 (42)
Unknown		1/1
Total	33/62 (53)	26/74 (35)

[a] Prevalence = number positive/total tested (%).

TABLE 2. Prevalence of *Bartonella henselae* antibody in 114 captive wild felids from California (USA).

Common name	Scientific name	Zoo[a]	Prevalence[b]	Titer
Cheetah	*Acinonyx jubatus*	SD	1/7 (14)	64
		SAC	1/4 (25)	64
Leopard	*Panthera pardus*	SD	3/11 (27)	64, 64, 64
		SAC	1/1 (100)	64
		MG	1/3 (33)	64
Snow leopard	*Panthera uncia*	SD	1/4 (25)	64
		MG	0/4 (0)	0
Tiger	*Panthera tigris*	SD	0/14 (0)	0
		SAC	0/1 (0)	0
		MG	1/1 (100)	128
Lion (African)	*Panthera leo*	SD	1/8 (12)	64
		SAC	0/1 (0)	0
		MG	0/1 (0)	0
Jaguar	*Panthera onca*	SD	1/5 (20)	64
Clouded leopard	*Neofelis nebulosa*	SD	1/3 (33)	128
		SAC	1/1 (100)	256
Mountain lion	*Felis concolor*	SD	0/2 (0)	0
		SAC	1/1 (100)	128
		MG	1/1 (100)	128
		Folsom	0/3 (0)	0
Jaguarundi	*Felis yagouaroundi*	SD	1/1 (100)	64
Caracal	*Felis caracal*	SD	1/1 (100)	64
Siberian lynx	*Felis lynx*	SD	0/1 (0)	0
Bobcat	*Felis rufus*	SAC	0/1 (0)	0
Serval	*Felis serval*	SD	1/3 (33)	64
		SAC	0/1 (0)	0
Fishing cat	*Felis viverrina*	SD	1/6 (17)	512
		SAC	0/1 (0)	0
Amur leopard cat	*Felis bengalensis*	SD	2/2 (100)	128, 64
Wild cat	*Felis silvestris*	SD	7/11 (64)	512, 512, 256 128, 128, 64, 64
Black-footed cat	*Felis nigripes*	SD	2/2 (100)	512, 128
		SAC	1/2 (50)	64
Geoffroy's cat	*Felis geoffroyi*	SAC	1/2 (50)	64
Margay cat	*Felis wiedii*	SAC	1/3 (33)	128
Jungle cat	*Felis chaus*	SAC	1/1 (100)	64

[a] SD = San Diego Zoo, SAC = Sacramento Zoo, MG = Micke Grove Zoo.
[b] Prevalence = number positive/total tested (%).

TABLE 3. Prevalence of *Bartonella henselae* antibodies by age group and species among 108 captive wild felids in California (USA).

Age (yr)	Prevalence[a] *Acinonyx* sp.	*Panthera* spp.	*Felis* spp.	Total
0–2	1/5 (20)	5/30 (17)	7/17 (41)	13/52 (25)
3–5	0/4 (0)	1/6 (17)	6/12 (50)	7/22 (32)
6–8	0/0 (0)	1/3 (33)	1/8 (25)	3/11 (27)
≥9	1/1 (100)	3/15 (20)	4/7 (57)	8/23 (35)

[a] Prevalence = number positive/total tested (%).

the cats 1- to 2-yr-old (10/27). The overall seroprevalence in cats ≥3-yr-old was 32% (18/56). Cats with the highest *B. henselae* antibody titers (≥1:256) were all <5-yr-old, and none of the cats >5-yr-old had antibody titers above 1:128. Cats of the genus *Felis,* aged 0- to 5-yr-old (13/29), were almost three times more likely to be *B. henselae* seropositive than the cats of the genera *Panthera* and *Acinonyx* (7/45) (RR = 2.88; 95% CI = 1.31, 6.36).

Among the San Diego Zoo cats with multiple blood samples tested, one male

cheetah was seropositive (1:64) at 11-yr-old, but negative 3 yr later. The other male cheetah was seronegative when tested at 7-yr-old, and was still negative 2 yr later. A male Persian leopard was positive at 9 yr-old (1:64) and remained positive at the same titer 3 yr later. A young female Indochinese tiger tested negative twice at 6 and 7 mo of age. A 6-yr-old male clouded leopard showed no change in titer (1:128) when tested three times over a 2 yr period, while a 7-yr-old female housed nearby, but not in direct contact with the male, was negative twice during the same period. A male fishing cat which had a high titer (1:512) when it was 4-yr-old had a lower titer (1:64) 3.5 yr later. At the Sacramento Zoo, only one female cheetah was tested more than once. It was positive at 1:64 when tested at 2 yr of age and had the same titer 1 yr later. The single free-roaming domestic cat from the San Diego Zoo had an antibody titer of 1:256 and was flea infested.

DISCUSSION

This is the first reported evidence of infection of free-ranging and captive wild felids with *Bartonella henselae* or a related species or subspecies of the genus *Bartonella* serologically cross-reactive with *B. henselae*. Previously, domestic cats were the only known reservoir of *B. henselae* (Regnery et al., 1992b; Koehler et al., 1994) and of *B. clarridgeiae* (Clarridge et al., 1995; Lawson and Collins, 1996). It will be important to determine if wild felids are infected by the same *Bartonella* spp. which infect domestic cats, or if their infection is caused by other species or subspecies which are closely related to *B. henselae* which allows serological detection with a *B. henselae* antigen. Infection of cats with *B. clarridgeiae* usually confers a cross-reactivity to *B. henselae* antigen (Kordick et al., 1977).

More than 50% of free-ranging bobcats from northern and southern California had *B. henselae* antibodies. Prevalence in free-ranging mountain lions was found to be lower and close to the overall prevalence in captive wild felids (30%) and domestic cats in the USA (Childs et al., 1995; Jameson et al., 1995). The higher prevalence in male mountain lions needs further investigation to determine which risk factor may be sex-associated.

In captive wild felids, antibody prevalence was much higher in small cats belonging to the genus *Felis* (47%) than in the larger cats belonging to the genus *Panthera* (17%) or *Acinonyx* (18%), which may explain in part the higher antibody prevalence at the Sacramento Zoo (40%) than at the San Diego Zoo (28.4%). At the Sacramento Zoo, 55% (11/20) of the animals tested belonged to the genus *Felis*, whereas this genus accounted for only 40% (32/81) of the wild cats tested at the San Diego Zoo. The antibody prevalence in small wild cats is also in the range of seroprevalence found in domestic cats in California (Chomel et al., 1995; Jameson et al., 1995).

Antibody prevalence to *B. henselae* fluctuated with age, on the contrary to what has been found in domestic cats (Childs et al., 1994). The highest prevalence was seen in the age group of 1- to 2-yr-old. This is similar to the report by Chomel et al. (1995) for domestic cats, which also had the highest antibody titers between 1 and 2-yr-old, suggesting that most of the infections must occur when the cats are reaching sexual maturity. Sexual maturity in the small wild cats is reached between 11 and 18 mo of age, whereas it occurs between 2 and 3 yr for most of the wild felids, especially the large cats (adult weight ≥20 kg) (Green, 1991).

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