

Seroepidemiology of *Bartonella vinsonii* subsp. *berkhoffii* Infection in California Coyotes, 1994-1998¹

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The prevalence of antibodies to *Bartonella vinsonii* subsp. *berkhoffii* in coyotes (*Canis latrans*) in California ranged from 51% in central to 34% in southern and 7% in northern California. Seropositive coyotes were more likely to be from coastal than inland counties ($p < 0.05$). The clustered distribution of *Bartonella* seropositivity in coyotes suggests that *B. vinsonii* subsp. *berkhoffii* infection is vectorborne. Further investigation is warranted to evaluate which arthropods are vectors and what the mode of transmission is from wildlife to domestic dogs and possibly humans.

Bartonella species are small gram-negative bacteria, which have been identified in a wide range of mammalian species, including felines and rodents (1-3). Several *Bartonella* species are human pathogens (4). Bacillary angiomatosis, initially recognized in AIDS patients, was related in the early 1990s to a new bacterium, *Bartonella henselae* (5,6), which was later associated with cat scratch disease (4). Clinical entities have been associated with several *Bartonella* species in humans; for example, *B. henselae*, *B. quintana*, and *B. elizabethae* have been associated with human cases of endocarditis (7-11). *B. vinsonii*, isolated only from small rodents, has not yet been confirmed as a cause of human disease (12). *B. vinsonii* subsp. *berkhoffii*, was recently isolated from a dog with endocarditis (13). It was found that 3.6% of 1,920 dogs in North Carolina and Virginia were seropositive to *B. vinsonii* subsp. *berkhoffii* antigen (14). *Bartonella*-seropositive dogs were 5.6 times more likely to be flea infested than were seronegative dogs, but 14 times more likely to have a history of heavy tick exposure. For the dogs with known tick exposure, a high

correlation was found between seroreactivity to *B. vinsonii* subsp. *berkhoffii* and seroreactivity to *Ehrlichia canis* and *Babesia canis*, both well-known tickborne infections. Furthermore, Breitschwerdt et al. (15) reported that dogs infected with *Ehrlichia* species were frequently coinfecting with *B. vinsonii* subsp. *berkhoffii*, suggesting that this infection in dogs could also be tickborne.

In July 1996, a 3 1/2-year-old boy was bitten by a coyote (*Canis latrans*) in Santa Clara County, CA, and became ill with fever and lymphadenopathy. The incident prompted an investigation of possible *Bartonella* infection in the boy and coyotes. Because of the clinical signs in the child and the absence of *Francisella tularensis* antibodies in two coyotes, the coyotes were tested for *Bartonella* antibodies by an immunofluorescence test with a *B. henselae* antigen, and both tested positive (titers of 1:128 and 1:512). *B. vinsonii* subsp. *berkhoffii*, identified by both polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) of the citrate synthase and 16S rRNA genes and by partial sequencing of the 16S rRNA gene, was isolated from several coyotes from Santa Clara County (3). The PCR/RFLP profiles of the coyote isolates and the domestic dog isolate were the same (ATCC 51672 strain).

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Dispatches

To determine the geographic distribution of *B. vinsonii* subsp. *berkhoffii* infection in California coyotes, we analyzed data for age, sex, and origin of the animals to establish possible risk factors associated with seropositivity and to identify high-risk coyote populations. Since coyotes (*C. latrans*) belong to the same genus as domestic dogs (*C. familiaris*), wild canids may serve as a reservoir of *B. vinsonii* subsp. *berkhoffii*, and transmission to domestic dogs could occur through common ectoparasites.

The Study

Coyote blood samples (nubuto filter strips or serum) collected from 34 of the 58 counties in California from 1994 to 1998 were tested serologically for *B. vinsonii* subsp. *berkhoffii* antibodies. Age, sex, county of sample collection, and collection date were recorded.

For 869 specimens, specific antibodies against a *B. vinsonii* subsp. *berkhoffii* purified antigen (outer membrane proteins) were detected by enzyme-linked immunosorbent assay (16). Cut-off values were established as described (17,18). The cut-off value (Optical Density [OD] > 0.2) for seropositivity was determined by the average OD plus three standard deviations (SD) of 76 nubuto strips from counties where all OD values were < 0.190. Statistically, animals with OD values > 0.2 can be considered to be seropositive with 99% confidence.

Univariate analysis by chi-square and Fisher exact tests was applied first to screen for factors associated with seropositivity to *B. vinsonii* subsp. *berkhoffii*. Potential confounders were evaluated by 10% change of estimates, i.e., odds ratio. Multiple logistic regression analysis was applied to evaluate the adjusted effects of the associated factors.

The overall prevalence of *B. vinsonii* subsp. *berkhoffii*-seropositive coyotes was 35% (95% confidence interval [CI]: 25% to 48%). *Bartonella* antibody prevalence varied from 51% in central California to 34% in southern and 7% in northern California (Table 1). The prevalence of seropositive coyotes from coastal counties was significantly higher than that of seropositive coyotes from inland counties (Figure) in all three geographic areas ($p < 0.05$). No gender difference in seropositivity was detected. Antibody prevalence was higher in adult coyotes (37%) than in young coyotes (< 1 year of age) (29%) (Table 2). The prevalence of high antibody titers (OD > 0.5) was

Table 1. Seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* infection in 869 coyotes, California, 1994–1998

Geographic region	County	No. sero-positive	(%)
Northern California		13/174	(7)
Coastal counties	Humboldt	2/12	(17)
	Mendocino	7/30	(23)
Inland counties	Siskiyou	0/26	(0)
	Modoc	0/47	(0)
	Lassen	1/7	(14)
	Plumas	1/40	(3)
	Butte	0/2	(0)
	Trinity	0/1	(0)
	Shasta	2/9	(22)
Central California		180/355	(51)
Coastal counties	Solano	0/4	(0)
	Sonoma	1/3	(33)
	Alameda	14/32	(44)
	Santa Clara	116/167	(69)
Inland counties	Santa Cruz	1/1	(100)
	Sierra	6/32	(19)
	Nevada	2/10	(20)
	Placer	0/1	(0)
	El Dorado	26/63	(41)
	Alpine	0/2	(0)
	Calaveras	0/2	(0)
	Tuolumne	4/8	(50)
	Mono	0/7	(0)
	Mariposa	4/7	(57)
Merced	2/2	(100)	
Sacramento	3/3	(100)	
Madera	1/11	(9)	
Southern California		115/340	(34)
Coastal counties	Monterey	4/10	(40)
	San Luis Obispo	18/56	(32)
	Santa Barbara	27/45	(60)
	Ventura	0/1	(0)
	Los Angeles	15/26	(58)
Inland counties	San Diego	6/6	(100)
	Kern	41/190	(22)
	San Bernardino	4/6	(67)

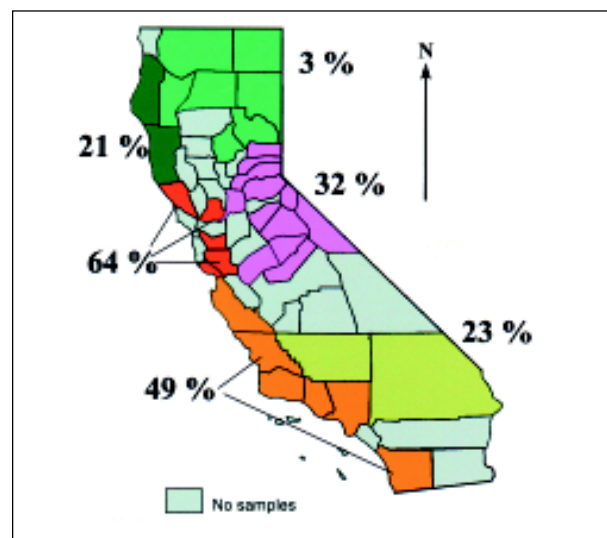


Figure. *Bartonella vinsonii* subsp. *berkhoffii* seroprevalence in 869 California coyotes (1994–1998).

Table 2. Seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* infection, by age, sex, and season, for 869 coyotes, California, 1994–1998

Characteristic	No. seropositive (%)
Age ^a	
<1 year old	35/122 (29)
≥1 year old	273/745 (37)
Sex ^b	
Female	155/407 (38)
Male	152/455 (33)
Season ^c	
Spring (Mar to May)	86/296 (29)
Summer (Jun to Aug)	121/286 (42)
Fall (Sep to Nov)	67/182 (37)
Winter (Dec to Feb)	27/85 (32)

^aNot available for two coyotes.

^bNot available for seven coyotes.

^cNot available for 20 coyotes.

Table 3. Annual *Bartonella vinsonii* subsp. *berkhoffii* antibody prevalence in 869 coyotes, by age group, California, 1994–1998

Year	All ages no. (%)	≥1 year old no. (%)	<1 year old no. (%)
1994	22/62 (35)	21/61 (34)	1/1 (100)
1995	55/159 (35)	53/146 (36)	2/13 (15)
1996 ^a	47/124 (37)	37/100 (37)	9/23 (39)
1997 ^a	115/275 (42)	107/238 (45)	8/36 (19)
1998	70/249 (28)	55/200 (28)	15/49 (31)

^aAge not available for one coyote.

similar in adults (20 [7.3%] of 273) and young coyotes (3 [8.6 %] of 35). There were no major differences in prevalence by age groups for each of the three following OD groups (OD > 0.2 and ≤ 0.3 [young coyotes, 37%; adults, 48%]; OD > 0.3 and ≤ 0.4 [young coyotes, 40%; adults, 27%]; and OD > 0.4 and ≤ 0.5 [young coyotes, 14%; adults; 19%]). Summer (June to August) had the highest prevalence (42%) of *Bartonella*-seropositive coyotes and spring had the lowest (29%) (Table 2). However, in young coyotes, antibody prevalence increased from 23% (3/13) in winter and 24% (6/25) in spring to 28% (12/43) in summer and 33% (13/39) in fall. Of the 25 coyotes < 6 months old, neither of the two captured in spring were seropositive, whereas 20% of the 10 captured in summer and 46% of the 13 captured in fall were seropositive.

Antibody prevalence was relatively constant during the 5-year period, from a low of 28% in 1998 to a high of 42% in 1997 (Table 3). In 1997, the prevalence was significantly higher in adults than in young coyotes (odds ratio [OR] = 2.86, 95% CI = 1.2, 7.5). This difference was associated with the high prevalence of positive adults from

coastal central (50 [82%] of 61) and southern (20 [77%] of 26) California. That year, none of the young coyotes from inland areas were seropositive, but 5 (62%) of 8 and 4 (80%) of 5 from coastal central and southern California were positive. Overall, infection occurred more frequently in young coyotes from coastal central and southern California (29 [57%] of 51) than from inland areas (5 [15%] of 34) (OR = 7.65, 95% CI = 2.3, 26.9).

By multiple logistic regression analysis, after adjustment for age, odds ratios for central California and southern California were 9.6 (95% CI: 5.2, 17.7) and 5.2 (95% CI: 2.8, 9.7) times higher, respectively, than for northern California. The adjusted odds ratio for the coastal counties compared with inland counties was 3.7 (95% CI: 2.7, 5.0). Similarly, coyotes ≥ 1 year old were 60% more likely to be seropositive (OR = 1.6, 95% CI = 1.0, 2.6) than young coyotes.

Conclusions

Because the discovery of *B. vinsonii* subsp. *berkhoffii* is so recent, little is known about the epidemiology and the mode of transmission of this organism in domestic dogs and wild canids. Of 54 coyotes from Santa Clara County, 16 (30%) were *B. vinsonii* subsp. *berkhoffii* bacteremic (19). Therefore, coyotes could serve as a potential reservoir for *B. vinsonii* subsp. *berkhoffii*, which could also be transmitted to domestic dogs, either by mechanical means (biting and scratching) or through arthropod vectors. *Bartonella* spp. are usually transmitted by arthropod vectors. Cats are the main reservoir for *B. henselae* and cat fleas are the main vector for transmission between cats (20,21). No direct transmission of *B. henselae* from cat to cat has been documented experimentally (22,23). Therefore, *B. vinsonii* subsp. *berkhoffii* transmission among coyotes and between coyotes and dogs is more likely to be vectorborne. Furthermore, the seasonal trends of *Bartonella* antibody prevalence in coyotes, especially young coyotes, indicate a moderate seasonal peak in summer and fall compared with winter and spring, when ectoparasites are the most abundant (14).

The high *Bartonella* antibody prevalence, especially in young coyotes, in coastal areas of central and southern California, indicates active transmission in these counties compared with the low prevalence of infection in young coyotes in inland areas. The clustered distribution of infection in young coyotes in California may be

associated with the mode of transmission of the infection, possibly through arthropods.

Limited data on the actual distribution of various arthropods throughout California are available. As suggested by Pappalardo et al. (14) and Breitschwerdt et al. (15), ticks could be potential vectors for *Bartonella* transmission in domestic dogs. The clustered distribution of *Bartonella* infection in California coyotes shown in this study seems to coincide with the known geographic distribution of tick species (*Ixodes pacificus* and *Dermacentor variabilis*) that can feed on carnivores in California (24). According to the studies of Chanotis et al. (25) and Ayala et al. (26), the distribution of sandflies in California is mainly in the Upper Sonoran zone of central California. This distribution could also match the coastal range of *Bartonella* infection in coyotes. Since sandflies are well-known vectors for *B. bacilliformis* transmission, they could also be considered for *Bartonella* spp. transmission in coyotes. Nevertheless, further studies need to be conducted to verify this hypothesis. In domestic carnivores, flea infestation is common and widespread in most of California. Cat fleas (*Ctenocephalides felis*) have been shown to be responsible for *B. henselae* infection in cats, and they also can infest domestic dogs and possibly coyotes. Although flea transmission cannot be completely ruled out, the dog study conducted in the Eastern United States showed that ten control dogs with heavy flea infestation but no known tick exposure had no seropositivity to *B. vinsonii* subsp. *berkhoffii* (14).

Only a few infectious agents can be transmitted by multiple vectors, e.g., tularemia and relapsing fever. Therefore, further investigation will be necessary to determine which of all the potential vectors mentioned above can harbor *B. vinsonii* subsp. *berkhoffii* under natural conditions and which one plays a major role for *B. vinsonii* subsp. *berkhoffii* transmission in canids. Furthermore, it is necessary to evaluate the risk of domestic dog infection with *Bartonella* spp. from wild canids and the potential risk of transmission to humans.

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Dr. Chang is a doctoral student in Epidemiology at the University of California, Davis, under the direction of Dr. Bruno B. Chomel. His research interests include molecular epidemiology of *Bartonella* infections and potential vectors for *Bartonella* spp. transmission.

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