

***Bartonella* spp. Isolated from Wild and Domestic Ruminants in North America¹**

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Bartonella species were isolated from 49% of 128 cattle from California and Oklahoma, 90% of 42 mule deer from California, and 15% of 100 elk from California and Oregon. Isolates from all 63 cattle, 14 deer, and 1 elk had the same polymerase chain reaction/restriction fragment length polymorphism profiles. Our findings indicate potential for inter- and intraspecies transmission among ruminants, as well as risk that these *Bartonella* spp. could act as zoonotic agents.

Bartonella species have been identified as important zoonotic agents (1,2). Cats are the main reservoir of *Bartonella henselae*, the agent that causes cat scratch disease in humans (1). Long-term bacteremia in cats and flea transmission from cat to cat, as confirmed by experimental infection, support a vectorborne transmission (3). Some human cases of cat scratch disease were not associated with any known exposure to cats (4), suggesting that other animal species may serve as reservoirs of *Bartonella*. Recently, new *Bartonella* species have been isolated from a wide range of mammals, including rodents (5-10), lagomorphs (11), carnivores (12-14), and cervids (14,15). Similarly, 90% of 42 mule deer (*Odocoileus hemionus*) from California were bacteremic with *Bartonella* isolates that were similar to isolates from roe deer in France (15) by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) of the 16S

rRNA and citrate synthase genes (14). Modes of transmission in these ruminants need to be established. Tick transmission has been suspected but not yet proven for dogs infected with *B. vinsonii* subsp. *berkhoffii* (16). Since fleas are less likely than ticks to infest cattle (17), ticks may play an important role in the transmission of *Bartonella* species from wild ruminants.

Our objectives were to determine if elk (*Cervus elaphus*), bighorn sheep (*Ovis canadensis*), and domestic cattle (*Bos taurus*) are infected with *Bartonella* and to determine the molecular relationships between *Bartonella* isolated from cattle and wild ruminants. We performed a cross-sectional study to compare the prevalence of *Bartonella* infection in a beef cattle herd in the California Sierra Nevada foothills and a dairy herd from the California Central Valley.

The Study

In February 1997, 42 samples from free-ranging mule deer were obtained from the Round Valley population, Mono and Inyo counties, California. In November 1997, 84 samples were

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collected from bighorn sheep herds in California and New Mexico. During January and February 1998, 100 blood samples were collected from elk in California and Oregon. One hundred twenty-eight cattle samples were collected: 12 from Oklahoma beef cattle in April 1998 and 116 from two California herds from May to July 1998. Fifty-three samples were collected from a >4,000-head beef cattle herd in the Sierra Nevada foothills and 63 samples from a >1,500-head dairy herd in the Central Valley. These 116 cattle were all > 2 years of age. Blood samples collected into lysis-centrifugation tubes were plated within 48 hours. Blood samples collected into EDTA tubes were frozen at -70° until plated. Wildlife and domestic herds were selected on the basis of ongoing surveys by the California and Oregon Departments of Fish and Game and researchers at the Universities of California and Oklahoma.²

Blood samples were cultured on heart infusion agar containing 5% rabbit blood and incubated in 5% CO₂ at 35°C for at least 4 weeks (18). Gram staining and biochemical tests were performed on representative isolates, which were defined as isolates with a unique PCR/RFLP profile for each of the three ruminant species. Nine representative isolates were identified, including one cattle strain (pattern I), five deer strains (patterns I, II, IV, V, and VI), and three elk strains (patterns I, II, and III). Standard methods were used to test for various preformed enzymes and carbohydrate use. Preformed bacterial enzyme activity was tested by Microscan Rapid Anaerobe Panel (Dade International Inc., West Sacramento, CA) (19).

An approximately 400-bp fragment of the citrate synthase gene was amplified as described (20). The amplified product was digested with *TaqI* and *HhaI* and *MseI* restriction endonucleases and visualized by gel electrophoresis. Banding patterns were compared with *B. henselae* (strain U-4; University of California, Davis, CA).

Cellular fatty acid composition was analyzed for representative cattle, deer, and elk isolates. Isolates were grown on rabbit blood agar at 35°C for 5 days. Fatty acid methyl ester derivatives were separated on a Hewlett-Packard series II 5890 gas chromatograph.

The PCR products used for DNA sequencing were purified with Microcon centrifugal filter devices (Millipore Corp., Bedford, MA) and sequenced with a fluorescent-based automated sequencing system. Primer BhCS.1137n (5'-AATGCAAAAAGAACAGTAAACA-3') (20) was used for partial sequencing of the 400-bp product of the citrate synthase gene. Nine representative strains from ruminants and one *B. henselae* strain (strain U-4, University of California, Davis) were sequenced. The GAP program of GCG software (Wisconsin Sequence Analysis Package, Genetics Computer Group, version 10) was used for alignments and comparisons of sequences, based on the 276 bp of the citrate synthase gene.

Using Epi Info version 6.03, we performed a chi-square test to assess association between prevalence of bacteremia of *Bartonella* infection and herd location. The *Bartonella* infection prevalence ratio (PR) was calculated to show the proportionate increase of infection prevalence due to difference in herd location.

Results

Bartonella spp. were isolated from 5 (42%) of 12 Oklahoma cattle, 58 (50%) of 116 California cattle, 38 (90%) of 42 California mule deer, 15 (15%) of 100 elk, and none of 84 bighorn sheep. In the California beef cattle herd, 25 (96%) of 26 bulls and 22 (81%) of 27 cows were *Bartonella* bacteremic; in the dairy herd, 11 (17%) of 63 cows were bacteremic. *Bartonella* bacteremia prevalence in the Sierra Nevada foothills beef cattle herd was therefore significantly higher than in the Central Valley dairy cattle herd (PR = 5.1; 95% confidence interval [CI] = 2.9-8.8). Prevalence of *Bartonella* bacteremic cows in the foothills herd was also significantly higher (81% vs. 17%) than in the Central Valley dairy cattle herd (PR = 4.7; 95% CI = 2.7-8.2). For elk, bacteremia prevalence differed significantly ($p = 0.0002$) between California (0 of 47) and Oregon (15 [28%] of 53). No *Bartonella*-bacteremic elk were found in the two California herds, but 11 (38%) of 29 elk from southwestern Oregon and 4 (17%) of 24 elk from northwestern Oregon were bacteremic.

The organisms isolated were short, slender gram-negative rods. By measuring preformed

²Collection sites for bighorn sheep were the Peninsular Ranges in California and the San Francisco River, Turkey Creek, and Red Rock in New Mexico. For elk, collection sites were the San Luis National Wildlife Refuge in Merced County and the Tupman Tule Elk State Reserve in Kern County (California); the Roseburg, Drain, and Demet herds, Douglas County (southwestern Oregon); and the Jewell Wildlife Area, Clatsop County (northwestern Oregon).

Dispatches

enzymes (Rapid Anaerobe Panel), the tested strains were found to be biochemically inert except for the production of peptidases, characteristic of the *Bartonella* profile (10077640).

Several strain profiles were observed by PCR/RFLP of the citrate synthase gene, using *TaqI* and *HhaI* and *MseI* endonucleases for deer (five profiles) and elk (three profiles) isolates (Figure). Conversely, all 63 cattle isolates had the same PCR/RFLP profile (Figure) with the same restriction enzymes. Overall, six different PCR/RFLP profiles were obtained from *Bartonella* isolated from cattle, deer, and elk. *Bartonella* isolated from cattle (63 of 63 tested; lanes 2, 12, and 22), mule deer (14 of 38 tested; lanes 3, 13, and 23), and an elk from southwestern Oregon (1 of 11 tested; lanes 10, 20, and 30) yielded the same PCR/RFLP profile (pattern I) with the three enzymes used. A second profile (pattern II) was obtained for *Bartonella* isolated from elk captured in northwestern Oregon (4 of 4 tested; lanes 8, 18, and 28) and from mule deer (5 of 38 tested; lanes 4, 14, and 24). A third profile (pattern III) was obtained for 10 of the 11 *Bartonella* isolated from elk captured in southwestern Oregon (lanes 9, 19, and 29). The other three profiles (patterns IV, V, and VI) were obtained for *Bartonella* isolated from mule deer ([pattern IV: 12 of 38 tested; lanes 6, 16, and 26];

[pattern V: 5 of 38 tested; lanes 5, 15, and 25]; and [pattern VI: 2 of 38 tested; lanes 7, 17, and 27]).

The cellular fatty acid composition was characteristic of the *Bartonella* genus for all isolates. The main fatty acids observed for the cattle, deer, and elk strains were octadecanoic acid (C_{18:1}, 45%-66%), octadecanoic acid (C_{18:0}, 12%-23%), and hexadecanoic acid (C_{16:0}, 13%-20%).

After pairwise comparisons, the partial sequencing analysis (276 bp) of the citrate synthase gene for the nine representative ruminant strains showed a high percentage of DNA similarity, from 93.12% to 100% (Table 1). The strains cattle-1, deer-1, and elk-1 belonging to the PCR/RFLP pattern I had 95.65% to 99.64% DNA similarity. The strains deer-2 and elk-2 with PCR/RFLP pattern II had 100% DNA similarity. The strain deer-1 with PCR/RFLP pattern I was closely related (98.91% DNA identity) to the strain deer-2 with PCR/RFLP pattern II. For strains deer-4 and deer-5, corresponding to PCR/RFLP patterns IV and V (similar digestion profiles with *HhaI* and *MseI* endonucleases and different profiles from *TaqI* endonuclease), a 98.55% DNA similarity was observed. Partial sequence analysis (276 bp) of the citrate synthase gene showed that all strains from ruminants were closely related to *B. weissii*, a *Bartonella* species isolated from domestic cats (Table 2).

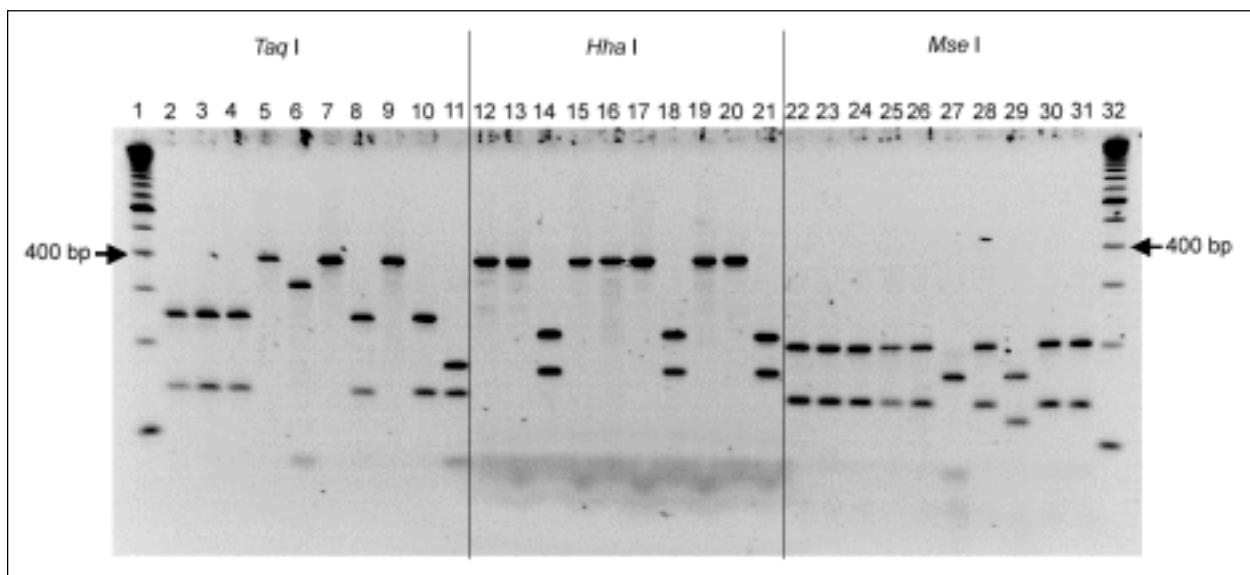


Figure. Polymerase chain reaction/restriction fragment length polymorphism of the citrate synthase gene of isolates from cattle, deer, and elk, with *TaqI*, *HhaI*, and *MseI* endonucleases. Lanes 1 and 32, standard 100-bp molecular ladder; lanes 2, 12, and 22, cattle isolate; lanes 3 to 7, 13 to 17, and 23 to 27, deer isolates; lanes 8 to 10, 18 to 20, and 28 to 30, elk isolates; lanes 11, 21, and 31, *B. henselae* strain.

Dispatches

Table 1. DNA similarity values and GenBank accession numbers based on 276 bp of the citrate synthase gene of the nine representative ruminant strains

Organism/ accession no.	% Similarity by strain								
	Cattle-1	Deer-1	Deer-2	Deer-4	Deer-5	Deer-6	Elk-1	Elk-2	Elk-3
Cattle-1 AF228768	100.00	95.65	96.01	94.57	94.57	94.57	99.64	96.01	94.57
Deer-1 AF228769	-	100.00	98.91	93.84	93.84	93.12	95.65	98.91	93.84
Deer-2 AF228771	-	-	100.00	94.20	94.20	93.48	96.01	100.00	94.20
Deer-4 AF228774	-	-	-	100.00	98.55	94.93	94.57	94.20	94.57
Deer-5 AF228775	-	-	-	-	100.00	94.20	94.57	94.20	94.57
Deer-6 AF228776	-	-	-	-	-	100.00	94.20	93.48	96.01
Elk-1 AF228770	-	-	-	-	-	-	100.00	96.01	94.57
Elk-2 AF228772	-	-	-	-	-	-	-	100.00	94.20
Elk-3 AF228773	-	-	-	-	-	-	-	-	100.00

Table 2. DNA similarity values based on 276 bp of the citrate synthase gene of the nine ruminant strains compared with those of the *Bartonella* strains in GenBank

Organism/accession no.	% Similarity by strain								
	Cattle-1	Deer-1	Deer-2	Deer-4	Deer-5	Deer-6	Elk-1	Elk-2	Elk-3
<i>B. bacilliformis</i> U28076	86.59	87.68	87.32	84.78	85.51	84.78	86.59	87.32	87.68
<i>B. grahamii</i> Z70016	90.22	90.22	90.58	91.67	90.22	90.58	90.58	90.58	89.49
<i>B. taylorii</i> Z70013	88.41	87.32	86.96	87.68	87.68	87.68	88.04	86.96	88.04
<i>B. tribocorum</i> AJ005494	89.86	89.13	89.49	90.58	89.13	88.41	89.49	89.49	88.04
<i>B. doshiae</i> Z70017	88.41	86.59	86.96	86.96	86.23	87.68	88.04	86.96	85.87
<i>B. vinsonii</i> subsp. <i>vinsonii</i> U28074	88.69	89.05	87.96	88.69	88.69	89.42	88.32	87.96	87.96
<i>B. vinsonii</i> subsp. <i>berkhoffii</i> U28075	89.86	89.49	89.13	87.68	87.68	88.41	89.49	89.13	86.96
<i>B. vinsonii</i> subsp. <i>arupensis</i> U77057	90.22	89.13	89.13	90.94	90.94	90.22	89.86	89.13	88.77
<i>B. weissii</i> AF071190	99.64	95.65	96.01	94.57	94.57	94.20	100.00	96.01	94.57
<i>B. clarridgeiae</i> U84386	90.58	89.49	89.86	88.77	88.04	88.77	90.22	89.86	89.49
<i>B. henselae</i> strain U-4	90.58	88.41	89.49	88.77	88.77	87.32	90.22	89.49	87.68
<i>B. henselae</i> strain Houston-1 L38987	90.58	88.41	89.49	88.77	88.77	87.32	90.22	89.49	87.68
<i>B. koehlerae</i> AF176091	89.13	88.41	88.77	89.49	88.77	87.32	88.77	88.77	87.32
<i>B. quintana</i> Z70014	90.22	88.04	88.41	87.68	86.96	88.41	89.86	88.41	88.77
<i>B. elizabethae</i> Z70009	88.41	88.04	88.41	90.22	88.77	89.49	88.04	88.41	88.04
Strain C7-rat Z70020	88.41	88.04	88.41	90.22	88.77	89.49	88.04	88.41	88.04
Strain C5-rat Z70018	88.77	88.77	89.13	90.22	88.77	87.32	88.77	89.13	87.68
Strain C4-phy Z70019	87.32	86.23	86.96	86.96	86.96	86.59	86.96	86.96	85.15
Strain C1-phy Z70022	86.59	85.51	86.23	86.23	86.23	85.87	86.23	86.23	84.42
Strain R-phy2 Z70011	87.32	86.23	86.96	86.96	86.96	86.59	86.96	86.96	85.15
Strain R-phy1 Z70010	88.04	87.68	88.04	87.32	87.32	87.32	87.68	88.04	85.87
Strain N40 Z70012	90.22	88.77	89.13	88.77	87.32	87.32	89.86	89.13	86.96
Strain A1 U84372	88.77	87.68	88.77	88.04	87.32	88.04	88.41	88.77	86.23
Strain A2 U84373	88.41	87.32	88.41	87.68	87.68	87.68	88.04	88.41	86.23
Strain A3 U84374	88.77	88.04	88.77	88.04	88.04	88.04	88.41	88.77	86.23
Strain B1 U84375	88.49	89.13	88.77	88.77	88.77	89.49	89.13	88.77	88.04
Strain B2 U84376	89.86	89.49	89.13	88.41	88.41	89.13	89.49	89.13	87.68
Strain C1 U84377	88.77	89.13	88.77	87.68	86.96	88.41	88.41	88.77	86.59
Strain C2 U84378	88.77	89.13	88.77	87.68	86.96	88.41	88.41	88.77	86.59
Strain D1 U84379	89.86	88.77	88.77	90.58	90.58	89.86	89.49	88.77	88.41
Strain D2 U84380	90.22	89.13	89.13	90.94	90.94	90.22	89.86	89.13	88.41
Strain D3 U84381	90.58	89.86	89.49	90.58	90.58	90.58	90.22	89.49	88.77
Strain D4 U84382	90.22	89.13	89.13	90.94	90.94	90.22	89.86	89.13	88.77
Strain D5 U84383	89.49	89.13	88.41	90.22	90.22	89.49	89.13	88.41	88.04
Strain D6 U84384	90.58	89.86	89.49	90.58	90.58	90.58	90.22	89.49	88.77
Strain D7 U84385	90.22	89.13	89.13	90.94	90.94	90.22	89.86	89.13	88.41

Conclusion

This is the first published report of isolation of *Bartonella* spp. from free-ranging wild ruminants and domestic ruminants in North America. Our results suggest that deer, elk, and domestic cattle are possible reservoirs of *Bartonella* spp. Selected bighorn sheep populations from California and New Mexico appeared to be free of *Bartonella*. The first report of infection of cattle with a *Bartonella* organism was made in 1934 by Donatien and Lestoquard, who proposed the name *B. bovis* or *Haemobartonella bovis* (21). In 1942, Lotze and Yiengst also described *Bartonella*-like structures in American cattle (22); however, their identifications of *Bartonella*-like structures were based only on the morphologic aspects of these organisms in red blood cells also infected with *Theileria* or *Anaplasma*, two well-known tickborne infections.

Partial sequencing analysis of the citrate synthase gene of the ruminant strains showed that they were all closely related to each other and to a feline strain, *B. weissii*. Further studies by DNA-DNA hybridization may determine if these strains are specific to ruminants but closely related to *B. weissii*, or if they are in fact *B. weissii*. If the ruminant strains are identical to *B. weissii*, the high prevalence (89%) of *Bartonella* bacteremia observed in beef cattle may indicate that ruminants are the main reservoirs of *B. weissii*, which is not commonly isolated from cats.

The prevalence of *Bartonella* bacteremia was high in beef cattle and mule deer, possibly related to exposure to potential vectors. Since fleas are rarely observed on cattle and tick infestation is common in both cattle and deer, ticks are a possible source of infection for ruminants (17). Furthermore, *Bartonella* DNA has recently been demonstrated in a high percentage of ticks infesting roe deer in Europe (23,24). The herd of beef cattle from the Sierra Nevada foothills, where tick infestation is common, has permanent access to open pastures. In contrast, the dairy cattle herd from the Central Valley has little or no access to pastures and tick infestations are not commonly observed (R. BonDurant, pers. comm.). Therefore, geographic differences in the prevalence of *Bartonella* infection in California cattle herds warrant further investigation for possible tick transmission of *Bartonella* spp. among these animals.

PCR/RFLP analysis of the citrate synthase gene has been widely used for identification of *Bartonella* organisms to the species level (25-27). We identified one PCR/RFLP profile for all the cattle isolates, but several profiles for deer and elk. This diversity by geographic location is of epidemiologic interest and warrants further investigation. Only one elk from southwestern Oregon had a strain with a similar PCR/RFLP profile to that of domestic cattle, suggesting that wild ruminants could be infected with *Bartonella* species that are not commonly shared with cattle.

Our findings also suggest that transmission of *Bartonella* may occur among cattle and wildlife, especially mule deer, which are more abundant in the western USA than elk and are more likely to be sympatric with cattle. Collection and analysis of ticks on wild animals and cattle and from the environment will be necessary to determine if ticks can be infected with *Bartonella* species. Whether *Bartonella* isolated from these ruminants are human pathogens is still unclear. The recent report of a cattle rancher who was infected with a new *B. vinsonii* subspecies (28) warrants further investigation to establish if these *Bartonella* species could be zoonotic and whether humans could potentially be infected by tick bites during work or recreation.

Dr. Chang is pursuing his Ph.D. in epidemiology at the University of California, Davis, under the direction of Bruno B. Chomel. His research interests include epidemiology of zoonoses, especially the molecular epidemiology of *Bartonella* infections and potential vectors for *Bartonella* spp. transmission.

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