Dispatches

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

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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. berkoffii* (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
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 Hhal and MseI restriction endonucleases and visualized by gel electrophoresis. Banding patterns were compared with 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

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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).

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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 (C18:1, 45%-66%), octadecanoic acid (C18:0, 12%-23%), and hexadecanoic acid (C16:0, 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.](image-url)
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  | 93.84  | 94.57  | 94.20 | 94.20 | 94.20 |
| Deer-4 AF228774        | -        | -      | -      | 100.00 | 94.57  | 94.57  | 94.57 | 94.57 | 94.57 |
| Deer-5 AF228775        | -        | -      | -      | -      | 100.00 | 94.20  | 94.57 | 94.20 | 94.20 |
| Deer-6 AF228776        | -        | -      | -      | -      | -      | 100.00 | 94.20 | 94.20 | 94.20 |
| 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 |
| *B. bacilliformis* U28076 | 86.59   | 87.68  | 87.32  | 84.78  | 85.51  | 84.78  | 86.59 | 87.32 | 87.68 |
| *B. grahamii* Z70016    | 90.22   | 90.22  | 90.58  | 91.67  | 90.58  | 90.58  | 90.58 | 90.58 | 89.49 |
| *B. taylorii* Z70013    | 88.41   | 87.32  | 86.96  | 87.68  | 87.68  | 88.04  | 89.66 | 88.04 | 88.04 |
| *B. tribocorum* AJ005494 | 89.96   | 89.13  | 89.49  | 90.58  | 89.13  | 89.49  | 89.49 | 89.49 | 89.49 |
| *B. vinsonii* subsp. vinsonii U28074 | 88.69   | 89.05  | 87.96  | 88.69  | 89.42  | 89.42  | 87.96 | 87.96 | 87.96 |
| *B. vinsonii* subsp. berkhoffii U28075 | 89.69   | 89.49  | 89.13  | 87.68  | 87.68  | 88.41  | 89.49 | 89.13 | 89.49 |
| *B. vinsonii* subsp. arupensis U77057 | 90.22   | 89.13  | 89.13  | 90.94  | 90.94  | 90.22  | 89.86 | 89.13 | 89.86 |
| *B. weissii* AF071190   | 99.66   | 95.65  | 96.01  | 94.57  | 94.57  | 94.20  | 100.00 | 96.01 | 94.57 |
| *B. clarridgeiae* U8436 | 90.58   | 89.49  | 89.86  | 88.77  | 88.77  | 90.22  | 89.86 | 89.49 | 89.49 |
| *B. koehlerae* strain C7-rat Z70020 | 88.41   | 88.04  | 88.41  | 88.77  | 88.77  | 87.32  | 90.22  | 89.49 | 89.49 |
| *B. henselae* strain C1-phy Z70019 | 86.59   | 85.51  | 86.23  | 86.23  | 86.23  | 85.87  | 86.23  | 86.23  | 86.23  |
| *B. elizabethae* strain C1 U84377 | 88.77   | 89.13  | 89.13  | 90.94  | 90.94  | 89.86  | 89.13  | 89.13  | 89.13  |
| *B. quintana* strain D1 U84379 | 87.32   | 86.23  | 86.23  | 86.23  | 86.23  | 85.87  | 86.23  | 86.23  | 86.23  |
| *B. henselae* strain Houston-1 L38987 | 87.32   | 86.23  | 86.23  | 86.23  | 86.23  | 85.87  | 86.23  | 86.23  | 86.23  |
| *B. koehlerae* strain C7-rat Z70020 | 88.41   | 88.04  | 88.41  | 88.77  | 88.77  | 87.32  | 90.22  | 89.49 | 89.49 |
| *B. koehlerae* strain C1-phy Z70019 | 86.59   | 85.51  | 86.23  | 86.23  | 86.23  | 85.87  | 86.23  | 86.23  | 86.23  |
| *B. elizabethae* strain C1 U84377 | 88.77   | 89.13  | 89.13  | 90.94  | 90.94  | 89.86  | 89.13  | 89.13  | 89.13  |
| *B. clarridgeiae* U8436 | 90.58   | 89.49  | 89.86  | 88.77  | 88.77  | 90.22  | 89.86 | 89.49 | 89.49 |
| *B. koehlerae* strain C7-rat Z70020 | 88.41   | 88.04  | 88.41  | 88.77  | 88.77  | 87.32  | 90.22  | 89.49 | 89.49 |
| *B. henselae* strain Houston-1 L38987 | 87.32   | 86.23  | 86.23  | 86.23  | 86.23  | 85.87  | 86.23  | 86.23  | 86.23  |
| *B. henselae* strain Houston-1 L38987 | 88.04   | 87.68  | 88.04  | 87.32  | 87.32  | 87.32  | 87.68  | 88.04 | 85.87  |
| *B. henselae* strain Houston-1 L38987 | 90.22   | 88.77  | 89.13  | 88.77  | 88.77  | 87.32  | 89.13  | 88.77 | 86.96  |
| *B. clarridgeiae* U8436 | 89.13   | 88.41  | 89.86  | 89.49  | 89.49  | 87.32  | 89.13  | 88.77 | 86.96  |
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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