IDENTIFICATION OF A NOVEL STRAIN OF *BORRELIA HERMSII* IN A PREVIOUSLY UNDESCRIBED NORTHERN CALIFORNIA FOCUS

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Abstract. An epizootiologic investigation testing for the presence of tick-borne relapsing fever spirochetes in rodent and tick hosts was conducted at Eagle Lake in Lassen County, California. Six of 211 *O. hermsi* ticks and none of 180 rodents were polymerase chain reaction (PCR)–positive for *Borrelia* spirochetes. Sequencing of the PCR-amplified flagellin gene fragment suggests that the spirochetes from Eagle Lake represent a previously undescribed strain of *Borrelia hermsii*.

Tick-borne relapsing fever is a zoonotic disease caused by spirochetes of the genus *Borrelia*. Three species of spirochete, each believed to have unique vertebrate and invertebrate hosts, are known agents of relapsing fever in North America. The spirochetes are named for specific Argasid tick vectors: *Borrelia hermsii* is transmitted by *Ornithodoros hermsi*, *B. parkeri* is transmitted by *O. parkeri*, and *B. turicata* is transmitted by *O. turicata.*1 Unique and well-defined ecologic conditions are believed to be associated with the presence of each of the relapsing fever spirochetes. In California, *B. hermsii* infects chipmunks and Douglas squirrels in forests above 1,000 meters while *B. parkeri* infects burrowing rodents found at lower elevations (*B. turicata* occurs only in the southwestern United States, Florida, and Mexico).2 Previous epidemiologic studies have shown a focal distribution of relapsing fever spirochetes in the high mountains of California.3 To further elucidate the geographic distribution of relapsing fever spirochetes in California, we chose to investigate the presence or absence of relapsing fever *Borrelia* in an area that has not previously been identified as a focus of tick-borne relapsing fever: Eagle Lake in Lassen County, California.

MATERIALS AND METHODS

Eagle Lake lies at the juncture of four major ecologic provinces in northeastern California, 26 miles north of Susanville. The lake is bordered on the west by the Cascade range, on the south by the Sierra Nevada range, on the east by the Great Basin, and on the north and east by the volcanic Modoc Plateau. The elevation at lake level is 1,900 meters and the surrounding forests rise as high as 3,000 meters. Field work for this study was performed at the University of California Nature Reserve System's Eagle Lake Field Station on the east shore of the lake. Habitats covered during trapping included sagebrush/juniper communities surrounding the field station and pine/fit forests in the adjacent hills. Trapping was carried out over a 10-day period in late June and a 10-day period in late August 1995. Only chipmunks (*Tamias* spp.) and Douglas squirrels (*Tamiasciurus douglasii*) were processed; other captured rodents (California ground squirrels, gray squirrels, and golden mantled ground squirrels) were released. Chipmunks were not categorized by species due to the reported difficulties in making accurate field identifications.4 Twenty wire mesh live traps were set in a north to south transect approximately 30 meters apart and 10 traps were set at random sites around the field station grounds.

Traps were baited with rolled oats and diced apple and were checked twice a day. Captured animals were transported to a processing station where they were anesthetized in a glass jar containing Metafoam® (Mallinckrodt Veterinary, Inc., Mundelein, IL) soaked cotton as previously recommended for use with wild rodents.5 Each animal’s sex, weight, snout to vent length, age class (based on size, weight, and primarily the smoothness or coarseness of the pelts), and species identification were recorded, and ectoparasites were collected and stored in 100% ethanol. Blood was drawn from the retro-orbital vein plexus and collected into 500-µl plastic microcentrifuge tubes. Blood samples were stored on ice in the field and at −20°C in the laboratory until DNA extraction was performed. Captured animals were marked with numbered metal ear tags. Recaptured animals were not bled again unless the previous collection was in June and the animal was recaptured in August. All animals were released at the site of capture. Rodent handling procedures were approved by and in accordance with the University of California Davis Animal Research Services guidelines.

Tick collections were performed during the same periods as the rodent trapping. Two techniques were used in the collection of *Ornithodoros* ticks. The first method involved placing metal pans containing dry ice at the suspected entrance to rodent dens and burrows. These traps were checked every 4 hr and ticks caught in the pan were collected and stored in 100% ethanol at −20°C. The second method involved identifying snags (the standing remains of dead trees) that contained active or abandoned rodent nests. The snags were then excavated and the debris was sifted in pans as described previously.6 Ticks collected from snags were stored as described earlier.

Spirochete DNA was isolated from rodent serum based on methods described previously.7 Precipitated DNA was resuspended in 20–30 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4) and 1–3 µl was used in each polymerase chain reaction (PCR). The primers used (forward: 5’-AGCTGGATCACAAGCTTCATGGACA-3’, reverse: 5’-CCCTCTATCTTTGCAAGTGACA-3’) were designed by Roger N. Picken (Department of Pathology, Loyola University Medical Center, Maywood, IL) and will amplify a region of the flagellin gene from all three species of tick-borne relapsing fever spirochetes.8 The PCR was carried out in an Idaho Technologies (Idaho Falls, ID) Air Cycler with the
following cycle parameters: 94°C for 15 sec (hot start), followed by 40 cycles of 93°C for 10 sec (denaturation), 52°C for 15 sec (annealing), and 72°C for 60 sec (extension), and a final extension at 72°C for 3 min following the last of the 40 cycles. Borrelia hermsii HS1 (ATCC 35210; American Type Culture Collection, Rockville, MD) and B. burgdorferi B31 (ATCC 35209) whole cells and DNA served as positive controls, respectively, for both extractions and PCR. Type Culture Collection, Rockville, MD) at a concentration of 10 mg/ml. Samples were then incubated at 37°C for an additional 20 min. The tubes were centrifuged at maximum speed in a microcentrifuge for 3 min to pellet the tick material and beads and the supernatant was transferred to a clean 1.5-ml tube. The supernatant was extracted with phenol:chloroform (1:1) and the DNA was precipitated with isopropanol. The DNA was resuspended in 10 μl of TE buffer and 1 μl was used in each PCR. The PCR cycle parameters were identical to those described earlier for all steps except for the annealing temperature: 48°C was used for DNA from ticks. All PCR products were electrophoresed on 1.5% agarose gels and visualized by staining with ethidium bromide. The PCR products were sequenced by the University of California Davis Molecular Biology Sequencing Service and sequences were analyzed using the Sequencher® (Gene Codes Corp., Ann Arbor, MI) computer program.

RESULTS

A total of 180 rodents, 168 Tamias species and 12 T. douglassi, were trapped and bled during the two collection periods. None of the blood samples tested were positive for the presence of spirochetes by PCR analysis (Table 1). The sensitivity of the DNA extraction methods and PCR cycle conditions used in this study were determined using whole dog blood samples spiked with serially diluted concentrations of spirochetes. These methods allowed the detection of spirochetes at a concentration of fewer than one spirochete per microliter of blood. An annealing temperature of 52°C was necessary to avoid amplification of nonspecific eukaryotic DNA.

Ornithodoros hermsi ticks were collected from 12 snags and 2–130 ticks were collected per snag. No O. hermsi ticks were collected from the dry ice traps but nine O. coriaceus ticks were found using this method. Dry ice traps were only used during the June collection period. Ticks were identified as O. hermsi based on several taxonomic features (Figure 1). A total of 211 O. hermsi and the nine O. coriaceus ticks, which have not been reported as hosts of relapsing fever spirochetes but are the vector of epizootic bovine abortion, were tested for the presence of spirochetes by the PCR (Table 2). Six of the O. hermsi, from four different snags, gave positive PCR results (Figure 2). None of the O. coriaceus was PCR positive. The forward and reverse DNA sequence of the PCR products showed 95% sequence homology with the previously published sequence for that region of the flagellin gene of two different strains of B. hermsii (Figure 3). The sequence divergence was due to a single nucleotide substitution at position 623 and a nine nucleotide deletion from position 610 to 618. The point mutation changes the amino acid encoded at that position from either a valine or glutamine to the other B. hermsii strains to an alanine. The nine base deletion removes three amino acids (a glutamine and two alanines) from the middle region of the flagellin protein but conserves the remainder of the reading frame.

DISCUSSION

Tick-borne relapsing fever is a reportable disease in the state of California, with 232 confirmed cases between 1970 and 1997 (Bissell SR, 1998, California Department of Health Services, Sacramento, CA, unpublished data). Most human infections are acquired when ticks are brought into homes or cabins by rodents establishing nests indoors, although some cases have involved no more than a day hike in the mountains as the source of exposure. The prevalence rate of B. hermsii in rodent hosts from the three main foci in California (Packer Lake, Big Bear Lake, and Lake Tahoe) has been reported at levels of approximately 7% based on microscopic analysis of blood smears from white mice inoculated with chipmunk or Douglas squirrel blood. A study of O. hermsi ticks conducted by Longanecker in 1951 found that 46% of O. hermsi-infested snags at Big Bear Lake and Lake Tahoe contained infected ticks and the prevalence for all ticks tested was 12.4%. The much lower prevalence discovered at Eagle Lake (33%) of infested snags had infected ticks and 2.8% of all ticks tested positive suggests that this region does not pose as significant a public health risk as the state’s main foci, but people maintaining and staying in camping and lodging facilities around the lake should still take precautions to rodent-proof their dwellings. A recent review of tick-borne relapsing fever cases in the northwestern United States and southwestern Canada concluded that tick-borne relapsing fever cases frequently go undiagnosed.

The discovery of O. hermsii-infected ticks at Eagle Lake should aid physicians in recognizing this illness in patients that have visited the area.
Infected *O. hermsi* ticks have been shown to maintain spirochetes in later instars at a frequency of 35–48%, and female ticks pass spirochetes onto their larvae via transovarial transmission at a minimum rate of 5.3%.

The ability of the *Borrelia* to persist in the ticks without passing through a vertebrate host and the absence of spirochetes from all of the rodents tested in this study reflects the importance of the ticks in supporting *B. hermsii*. The absence of PCR-positive rodents also illustrates the need to test invertebrate as well as vertebrate hosts when conducting epidemiologic surveys of vector-borne diseases.

The unique DNA sequence within the amplified region of the flagellin gene of the Eagle Lake *Borrelia* suggests that this is a novel strain of *B. hermsii*. The identification of the ticks in which the spirochetes were found as *O. hermsi* and the close sequence homology (95%) with other known strains of *B. hermsii* support this classification. The mutations within the flagellin sequence suggest that these spirochetes are unique from other isolates of *B. hermsii* from California and Washington. The study of Picken analyzed the ability of two different oligonucleotide probes to hybridize to a region within the PCR-amplified flagellin sequences of multiple strains of *B. hermsii*. Due to the fact that the YOR-1 PCR product shows greater sequence homology to HS1 than does the Eagle Lake *Borrelia* (Figure 3), it is unlikely that the Eagle Lake *Borrelia* would have a sequence identical to any of the strains that were detectable with this probe. The region in which the sequence divergence is seen has been identified as a variable region of the flagellin gene, at both the nucleotide and amino acid level, among members of the *Borrelia* genus. Picken identified a similar nine base deletion or possible duplication within this region that distinguishes the *B. burgdorferi* gene from that of *B. hermsi*.

This variability suggests that the nine base deletion described for the spirochetes found at Eagle Lake would not

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**Table 2**

Ticks tested for spirochetes*

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection period</th>
<th>Larvae (no. PCR +)</th>
<th>Nymph (no. PCR +)</th>
<th>Adult (no. PCR +)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ornithodoros hermsi</em></td>
<td>June</td>
<td>1 (0)</td>
<td>46 (1)</td>
<td>37 (0)</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>5 (0)</td>
<td>47 (1)</td>
<td>75 (4)</td>
</tr>
<tr>
<td><strong>Totals for both collection periods</strong></td>
<td></td>
<td>6 (0)</td>
<td>93 (2)</td>
<td>112 (4)</td>
</tr>
<tr>
<td><em>O. coriaceus</em></td>
<td>June</td>
<td>(0)</td>
<td>(0)</td>
<td>9 (0)</td>
</tr>
</tbody>
</table>

* PCR = polymerase chain reaction.
affect flagellar function and is simply a product of random genetic divergence, although the possibility of selection for a unique phenotypic trait cannot be ruled out.

The absence of PCR-detectable spirochetes in the blood of chipmunks and Douglas squirrels suggests the interesting possibility that the Eagle Lake strain of spirochete is found in animals other than the traditional *B. hermsii* hosts or that this strain inhabits tissues or fluid other than blood. There are no records of human cases of tick-borne relapsing fever in Lassen County (Bissell SR, 1998, California Department of Health Services, Sacramento, CA, unpublished data), although this study has shown the presence of *B. hermsii* infected ticks at Eagle Lake. Perhaps the lack of confirmed human cases is due to presentation of anomalous disease symptoms that escape detection or are misdiagnosed, as has been suggested for cases where relapsing fever may produce clinical symptoms analogous to Lyme disease.\(^8\) Further studies involving genetic characterization, culturing, and infection assays in rodent and tick hosts would provide important information concerning the biological and evolutionary relationship of the Eagle Lake *Borrelia* to the other relapsing fever *Borrelia*.

**Figure 2.** Amplified products of 282 basepairs from polymerase chain reaction (PCR)–positive ticks and the three species of relapsing fever spirochetes. Lane 1, *Ornithodoros hermsi* #3; lane 2, *O. hermsi* #94; lane 3, *O. hermsi* #97; lane 4, *O. hermsi* #120; lane 5, *O. hermsi* #136; lane 6, *O. hermsi* #122; lane 7, 100-basepair DNA marker; lane 8, *Borrelia hermsii* HS1; lane 9, *B. parkeri*; lane 10, *B. turicata*. *Ornithodoros hermsi* #122 gave positive results after three separate PCRs but did not yield a band when amplification was attempted for this or subsequent gels. DNA degradation likely occurred in this sample since the time of the initial positive results. Negative PCR controls (*B. burgdorferi* DNA and sterile water) were run with each set of PCRs but are not shown on this gel. Values on the left are in basepairs.

**Figure 3.** Nucleotide sequences of the polymerase chain reaction–amplified fragment from the Eagle Lake *Borrelia* (ELB) and the previously published sequences for *Borrelia hermsii* strains HS1 and YOR-1. Primer sequences are not included. A dash in the sequence indicates identity with the ELB sequence and the dots indicate the site of a nine-base deletion with respect to the HS1 and YOR-1 sequences.
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