Isolation and Characterization of *Borrelia hermsii* Associated with Two Foci of Tick-Borne Relapsing Fever in California

Curtis L. Fritz,1* Lawrence R. Bronson,1 Charles R. Smith,1 Martin E. Schriefer,2 James R. Tucker,1 and Tom G. Schwan3

California Department of Health Services, Sacramento, California; Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; and Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana

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Relapsing fever, caused by the spirochete *Borrelia hermsii* and transmitted by the soft tick *Ornithodoros hermsi*, is endemic in many rural mountainous areas of California. Between 1996 and 1998, 12 cases of relapsing fever associated with two exposure sites in northern California were investigated. Follow-up at exposure sites included collection of soft ticks and serum specimens from sylvatic rodents. Attempts to cultivate spirochetes were made through inoculation of patient blood into mice and by feeding *Ornithodoros* ticks on mice. Three isolates of *B. hermsii* were recovered from two blood specimens and one pool of ticks. The protein and plasmid profiles of the three isolates were comparable to those of previous *B. hermsii* isolates from the western United States. Western immunoblotting of patient sera demonstrated an expanding immunologic response to antigens within four distinct molecular weight regions by 3 to 4 weeks postonset. Antibody to *B. hermsii* was detected in sera from 4 of 11 yellow-pine chipmunks (*Tamias amoenus*); no other rodent species collected were seropositive.

Tick-borne relapsing fever (RF) is a zoonotic disease endemic throughout much of Africa, central Asia, and the Americas (22). RF has been recognized as a cause of human morbidity in the western United States since the early 20th century (12). A recent epidemiologic review of RF in the United States indicated that 450 cases in patients with a broad age range (<1 to 81 years) and of both sexes (52% males) were reported in 11 western states between 1977 and 2000 (7). Individual cases of RF are most commonly reported, but large outbreaks also occur (2, 5, 24). RF is characterized by recurring episodes of fever and nonspecific symptoms (e.g., myalgia, headache, and gastrointestinal illness), separated by several days of apparent recovery (22). Although most patients recover promptly following initiation of antibiotic therapy, severe symptoms (e.g., Jarisch-Herxheimer reactions) and, rarely, death can occur.

Three species of spirochete—*Borrelia hermsii*, *Borrelia turicatae*, and possibly, *Borrelia parkeri*—have been identified as agents of RF in the United States. The most important agent in the western United States is *B. hermsii*, which is transmitted through the bite of the argasid tick (*Ornithodoros hermsi*). *B. hermsii* is maintained in enzootic cycles that involve *O. hermsi* and small sylvatic rodents, principally chipmunks (*Tamias* spp.) and pine squirrels (*Tamiasciurus* spp.) above elevations of 1,000 m (6). Human infection typically occurs when infected ticks living in rodent nests within a human dwelling seek out an alternative host upon which to feed. Because *O. hermsi* is nocturnal and feeds quickly, in 20 to 90 min, RF patients rarely recall bites from these ticks.

Most RF cases are diagnosed and treated empirically on the basis of the clinical presentation and a history of sleeping in a rural mountain dwelling within 2 weeks of onsets. While the diagnosis may be confirmed by the observation of spirochetes in a patient’s blood smear, attempts to culture, isolate, and identify these spirochetes to the species level are rarely performed. In California, between 3 and 18 cases of RF were reported each year between 1985 and 2002 (California Department of Health Services, unpublished data). Few isolates of *B. hermsii* from California have been described, despite the frequent occurrence and wide distribution of human cases of *B. hermsii* infection reported in many areas of the state dating back to 1921 (3). The last reported isolation of *B. hermsii* from a California RF patient was in a resident of San Francisco who was infected while vacationing in Siskiyou County in 1964 (1). That isolate and two others recovered from California RF patients in the 1960s have been described previously (17, 18). Here we report on the results of investigations of recent RF outbreaks in California, describe the immunologic profiles of RF patients and rodents, and describe the isolation and characterization of the *B. hermsii* isolates from the RF patients and ticks associated with these outbreaks.

MATERIALS AND METHODS

**Investigations of outbreaks.** RF is a reportable disease in California. Between 1996 and 1998, the California Department of Health Services endeavored to investigate all outbreaks of RF occurring in California. For the purposes of this study, an RF fever outbreak was defined as two or more confirmed RF cases epidemiologically associated with a common place and a common time of exposure. A patient with a confirmed case of RF was defined as one who had a clinically compatible illness and for whom laboratory confirmation of RF could be obtained either by observation of spirochetes in a blood smear or by positivity by *B. hermsii* serology. A patient with suspected RF was defined as one who had a concurrent clinically compatible illness or a history of a clinically compatible illness that was epidemiologically linked to a confirmed case but that lacked laboratory confirmation.

*Corresponding author. Mailing address: Division of Communicable Disease Control, California Department of Health Services, MS 7307, P.O. Box 997413, Sacramento, CA 95899-7413. Phone: (916) 552-9730. Fax: (916) 552-9725. E-mail: cfritz@dhs.ca.gov.*
Environmental evaluations were conducted at each exposure site. Implicated buildings were evaluated inside and outside for signs of rodent activity and ingress (e.g., fecal deposits and gnaw holes). Tomahawk (Tomahawk Live Trap, Tomahawk, Wis.) and Sherman (Sherman Trap Co., Tallahassee, Fla.) traps were set overnight to collect small rodents in and around buildings. Blood samples were collected by cardiac puncture, and the animals were then released. Carbon dioxide traps were set inside overnight to collect argasid ticks.

**Patient serology.** All human sera were tested in the Diagnostic and Reference Laboratory (CLIA identification no. 08D0800233) of the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colo., by standardized diagnostic enzyme immunoassay (EIA) and Western blotting. Samples submitted for RF serology are routinely tested in parallel for antibodies against *B. hermsii* and *Borrelia burgdorferi* in order to document the specificity of the test. EIA was performed as described previously (24). Briefly, *B. hermsii*-coated wells (100 ng/well) were incubated with a 1:100 dilution of patient or control serum, and bound antibody was detected with goat anti-human immunoglobulin G (IgG) plus IgM conjugated with alkaline phosphatase. After substrate development, specimens with reactivities from >1 to <3 standard deviations above the mean for six negative controls were interpreted as equivocal, and specimens with reactivities >3 standard deviations above the mean for the negative controls were interpreted as positive. Specimens with equivocal or positive EIA reactivities were further tested in separate IgM and IgG Western immunoblot assays. Western blots, as described below, were probed with human serum (1:100) for 30 min, and bound IgM or IgG was detected with alkaline phosphatase-labeled goat anti-human IgM or IgG conjugate and an appropriate substrate (Kirkegaard & Perry Laboratories). Western blots were stained with Coomassie brilliant blue.

**Western blot analysis.** Whole-cell lysates were electrophoresed in one-dimensional acrylamide gels and blotted onto nitrocellulose membranes by using Towbin buffer (23) and a Trans-Blot Cell (Bio-Rad Laboratories) according to the instructions of the manufacturer. The membranes were blocked overnight at room temperature with TSE-Tween (50 mM Tris [pH 7.4], 150 mM NaCl, 5 mM EDTA, pH 7.4) and a Trans-Blot Cell (Bio-Rad Laboratories) according to the instructions of the manufacturer. DNA purification and analysis. Total DNA was purified from 500 ml of stationary-phase BSK-H cultures of spirochetes (21). DNA samples were examined by agarose gel electrophoresis with a Mini-Sub DNA Cell (Bio-Rad Laboratories). DNA samples were electrophoresed in 0.3% agarose gels with TBE buffer (90 mM Tris, 90 mM boric acid, 20 mM EDTA) to resolve the plasmids. Gels were run with ethidium bromide at 50 V for 5 min and then 12 V for 16 h, and the DNA was visualized by UV transillumination.

**RESULTS**

Between 1996 and 1998, 30 cases of RF were reported through the statewide surveillance system to the California Department of Health Services; 5 additional unreported cases were identified during the present study. Of the 35 total cases, 16 were associated with seven outbreaks. We describe here the results of investigations of three of these outbreaks at two different exposure sites.

**Site A.** In August 1996, patients 1 and 2 (Table 1) developed febrile illnesses approximately 1 week after spending 5 days at site A. Both patients experienced recurrent episodes of fever up to 105°F for 2 to 3 days, separated by 3 to 6 days of apparent recovery. Patient 3 developed chills and fever to 104°F in April

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**Table 1. Clinical and laboratory features of confirmed and suspect RF cases associated with two outbreak sites in California**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>MoY of onset</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Outbreak site</th>
<th>No. of febrile episodes</th>
<th>Blood smear result</th>
<th>No. of wk between onset and serum collection</th>
<th>Serology result by:</th>
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\( ^a \) Abbreviations: M, male; F, female; NI, not ill; ND, not done; Pos, positive; Equiv, equivocal; Neg, negative.
1997, 2 days after spending 5 days at site A. Patient 3 recovered within 48 h following initiation of antibiotic therapy and experienced a single febrile episode. In August 1997, three members of the same extended family (patients 4, 5, and 6) experienced the onset of similar febrile illnesses 3 to 6 days after visiting site A.

In addition to these six acute RF cases, exposure to *B. hermsii* was identified retrospectively among three relatives (patients 7, 8, and 9) of patient 3, all of whom had visited site A. Patients 7 and 8 both experienced febrile illnesses approximately 1 week after visiting site A in 1995 and 1996, respectively. Patient 9 visited site A at the same time as patients 3, 7, and 8 but recalled no subsequent illness.

Site A is a family-owned ranch in Siskiyou County approximately 15 mi south of the California-Oregon border, at the southwestern edge of Butte Valley. Located at an elevation of 1,300 m, the habitat is principally yellow pine-oak forest with big sagebrush, bitterbrush, and rabbitbrush mix. Visual inspections, rodent surveys, and tick collection were conducted at site A on three occasions in 1997. The initial investigation in April 1997 noted evidence of extensive activity by golden-mantled ground squirrels (*Spermophilus lateralis*) and yellow-pine chipmunks (*Tamias amoenus*) throughout the area surrounding the two guest cabins. Stick piles and feces of wood rats (*O. hermsi*) were observed in buildings and sheds adjacent to the cabins. Mouse feces were noted inside both cabins. Investigation of the sleeping quarters in one cabin revealed five dead deer mice and four nests within the box-spring mattress of one bed. Blood spots were identified on bedding from which two *O. hermsi* ticks, one fully engorged, were collected. Two additional *O. hermsi* ticks were found in the bedding of a second cabin.

A second investigation was performed at the site in August 1997. Golden-mantled ground squirrels had access to the kitchen in one cabin, and chipmunks moved freely in and out of the attic of the second cabin. Ground squirrels, chipmunks, and a wood rat were collected; and serum samples were obtained.

A third investigation was performed at the site in October 1997. Sherman traps and dry ice stations failed to collect rodents or argasid ticks in the main cabin. Two wood rats were captured and released following collection of blood samples. Two wood rat nests were noted in the attic above the master bedroom. A dead chipmunk was recovered from one nest. The rodent nests were dismantled, and the nest materials were thoroughly inspected. A total of 25 *O. hermsi* ticks were recovered from the nests by using a Berlese funnel.

**Site B.** In August 1998, a 48-year-old male (patient 10) was hospitalized with a febrile illness following a 5-day vacation at site B. A high concentration of spirochetes was observed in a Wright’s-stained smear of his blood. The patient experienced a severe Jarisch-Herxheimer reaction following initiation of treatment with ceftriaxone. Seven and 12 days after the onset of illness in patient 10, two male siblings (patients 11 and 12; ages 2 and 4 years, respectively) who had accompanied patient 10 to site B were hospitalized with similar febrile illnesses. High concentrations of spirochetes were observed in the blood smear of patient 11, but no spirochetes were observed in the blood smear of patient 12.

Site B was at a condominium complex in Placer County, approximately 6 mi west of Lake Tahoe, Calif. The condominium was one of six attached units in a single building. All three patients slept at least one night in a single bedroom at the condominium. Site B was inspected in August 1998. Carbon dioxide traps were placed under the foundation. The condominium had an open-beamed roof that may have allowed rodents to enter without obvious signs of access. All the rooms within the condominium were thoroughly inspected for rodent harborage and argasid ticks; however, no evidence of rodents or ticks was found. Prior to the evaluation by public health officials, rodent control efforts, laundering, and housecleaning had been done and may have contributed to the lack of any positive findings at this site.

**Serology.** The antibody responses against *B. hermsii* among the patients evolved and peaked during the first few weeks postinfection, as evidenced by EIA and Western immunoblotting (Table 1). Reactivity to immunodominant antigen regions with molecular masses of 66, 41 to 39, 32 to 28, and 21 to 18 kDa was observed in Western blots. GlpQ was shown to comigrate with flagellin at 40 kDa (Fig. 1), and patient antibody against this antigen was demonstrated in recombinant immunoblots (data not shown).

Blood samples were collected from nine patients during the acute phase of infection, within 1 month of disease onset. Samples collected from patients 10 to 12 within the first week of disease onset were negative by EIA and displayed minimal banding in immunoblots (e.g., Fig. 1, lane 1). Samples collected from patients 1 to 6 between 1 and 4 weeks after onset demonstrated increased reactivities by EIA (equivocal or positive results) and increased numbers of bands reactive for IgM and IgG on immunoblots (e.g., Fig. 1, lanes 2, 3, and 4); during this collection interval, immunoblots of samples from all patients were positive. The specificities of the antibodies from RF-seropositive patients were demonstrated by increased reactivities against *B. hermsii* compared to that against *B. burgdorferi* by EIA and Western blotting (data not shown). Acute- and convalescent-phase sera were collected from two patients (patients 4 and 5). Convalescent-phase samples, collected approximately 9 weeks postonset, demonstrated sustained IgM and IgG reactivities in Western blots (e.g., Fig. 1, lane 5). Antibody levels were greatly diminished in samples collected at later time points; samples collected a year or more after the onset of disease (patients 7 and 8; Fig. 1, lanes 7 and 6, respectively) were equivocal by EIA and displayed strong reactivities to a single band at 20 kDa and faint reactivities to several other bands in IgG immunoblots.

Blood samples were obtained from 33 rodents at site A: 14 *S. lateralis*, 11 *T. amoenus*, 5 *P. maniculatus*, 2 *N. fuscipes*, and 1 *Tamias senex* rodent species. Western blot analysis showed that 4 (36%) of the 11 *T. amoenus* chipmunks had antibodies to *B. hermsii* antigens and to heterologous GlpQ expressed in *E. coli* (Fig. 2). No antibodies to *B. hermsii* were detected in sera from the other rodent species.

**Identification and characterization of *B. hermsii* isolates.** Two isolates (isolates RAL and WAD) of *B. hermsii* were recovered from the blood of patients (patients 4 and 11, respectively), and one isolate (isolate SIS) originated from ticks. The two isolates from the human patients were established in pure culture only after laboratory mice (*Mus musculus*) were first inoculated with the spirochetic blood, as all attempts to
isolate the organisms in culture medium directly from the patients’ blood failed. SIS was isolated from the blood of a mouse on which 22 O. hermsi ticks collected from the wood rat nest at site A had fed.

The protein profiles of whole-cell lysates were typical for B. hermsii, but the profiles for isolates from patients infected in eastern Washington were different (Fig. 3A). Differences were present in the mid-20-kb range, in which the variable small proteins migrate. The isolates were identified as B. hermsii by reactivity with monoclonal antibody H9826, an antiflagellin antibody specific to this species (Fig. 3B). The plasmid profiles of the new isolates were also consistent with those for plasmids from other isolates of the same species originating from Washington (Fig. 4). Some variability in the sizes of the linear plasmids in the 22- to 26-kb range was evident.

DISCUSSION

This report describes the first isolation in more than 30 years of B. hermsii spirochetes from RF outbreaks in California. Until recently, isolation of Borrelia was hampered by lack of an effective cultivation protocol. In 1971, Kelly (9) described a liquid medium that supported the continuous cultivation of B. hermsii in vitro. In 1982, Burgdorfer and coworkers (4) reported on a Borrelia spirochete that had been detected in ticks, isolated in Kelly’s medium, and bound to antibodies from Lyme disease patients. Although interest in research on this new spirochetal disease spread rapidly throughout the biomedical community, the medium remains difficult to make and maintain. Hence, culture of blood samples from suspect bor-
the subsequent in vitro cultivation. However, inoculation of blood samples into mice can amplify the extent that inoculation directly into liquid medium fails. Delays reduce the viability of the spirochetes in the samples to search laboratories. In our experience, the resultant shipping relocations patients is limited to a few specialized clinical or research laboratories. In our experience, the resultant shipping delays reduce the viability of the spirochetes in the samples to the extent that inoculation directly into liquid medium fails. However, inoculation of blood samples into mice can amplify existing spirochete numbers and improve the success of subsequent in vitro cultivation.

The paucity of isolates of RF spirochetes is exacerbated by the difficulty of finding infected O. hermsii ticks in nature. Unlike the ixodid (hard) tick vectors of Lyme disease spirochetes, which can be collected in large numbers from vegetation, the argasid (soft) ticks which transmit B. hermsii confine themselves to rodent burrows, nests, and hard-to-find refugia except during brief nocturnal feedings. The collection of ticks during investigations of RF cases is rarely successful, even when a specific exposure location (e.g., the master bedroom) is known. Traps baited with CO₂ provide a theoretical means of enticing feeding behavior in soft ticks (13), but in practice these efforts are rarely rewarding. Ticks have occasionally been found in unlaundered bed linens which have been left in situ subsequent to the RF patients’ exposure. Also, as in site A in the present study, ticks may be recovered from their usual harborage in a rodent nest when the nest can be located.

Whole blood from RF patients is the most likely source of new isolates of B. hermsii. However, if spirochete densities are less than 10⁷ cells per ml, the bacteria can elude microscopic examination, the diagnosis may be missed, and culture will not be attempted. Spirochetemia is maximal during the febrile periods of RF; few to no spirochetes are observed in blood specimens collected during afebrile intervals. Smears of blood collected during inappropriate periods in the illness cycle offer little diagnostic benefit and, because they yield false-negative results, can delay accurate diagnosis and antimicrobial treatment.

Serology is an ancillary diagnostic option for patients with RF-compatible illness who have recovered, who are epidemiologically linked to a confirmed case or implicated exposure site, or for whom the only available specimen is blood collected during an afebrile period. The sensitivities of serologic assays for RF are dependent upon the kinetics of the immunologic response following infection. In the present study, antibody was not detected in patients 10, 11, and 12 during the first week of disease. (Although RF in patient 12 was not laboratory confirmed, his symptomatology, rapid response to antibiotic treatment, and strong epidemiologic link to patients 10 and 11 strongly suggested that he experienced a contemporaneous B. hermsii infection.) Sera collected from patients 1 to 6 at 2 to 4 weeks postonset were equivocal or positive by ELISA and positive by Western immunoblotting. At least eight immunoreactive antigens were commonly observed in IgG immunoblots from samples from patients 4, 5, and 6 collected between 4 and 9 weeks postonset. Sera collected from patients 7 and 8 a year or more after disease displayed equivocal reactivities by ELISA and negative reactivities by immunoblotting. While Western immunoblotting for RF is available only through selected research laboratories at present, the results of the present study indicate that it has promise for confirmation of the diagnosis of RF in patients with early disease (>1 week postonset) and treated and/or resolved disease (9 weeks postonset).

Antibody cross-reactivity against numerous proteins of B. hermsii and B. burgdorferi occurs (11, 15, 20) and may lead to false-positive laboratory results when the clinical and epidemiologic pretest assessment is inadequate. In the present study the pretest likelihood of RF among ill patients was high and
immunospecificity was documented by differential antibody reactivities against B. hermsii. In addition, reactivity to GlpQ has been shown to be a discriminator of these related spirochetal infections (14, 20); to date, GlpQ and its enzymatic activity have been found only in RF Borrelia (16). Use of this diagnostic approach provided further evidence of RF exposure in rodents collected in the field; 4 of 11 (36%) serum samples collected from chipmunks (T. amoenus) from site A were seropositive for lysates of B. hermsii and the heterologously produced GlpQ. Given the ephemeral presence of detectable spirochetes in the blood of infected animals, as in humans, examination of rodent sera for anti-GlpQ antibodies should offer a more specific surveillance tool for RF Borrelia ecology.

Removal and exclusion of rodents are the principal means of preventing additional RF cases at a building implicated as a site of RF transmission. At site A, T. amoenus chipmunks were frequently observed to enter and exit the attic of one cabin through holes at the roofline and to nest within the walls and attic. The same attic space contained at least two wood rat nests, suggesting that O. hermsi ticks live in or near rodent nests, elimination of rodents without removal of the nests may actually increase the risk of transmission to humans, as ticks seek out an alternative host upon which to feed. However, rodent nests are often in inaccessible spaces in the building and cannot be readily located. In the present study, the wood rat nest at site A was not discovered until the third site investigation and after at least seven cases of RF had been acquired.

RF remains a cause of morbidity for visitors to the mountains of the western United States. Because of the often self-limited clinical course and rapid response to empirical antibiotics, many cases of RF may go undiagnosed and unreported. However, RF can be severe, with neurologic, respiratory, and cardiovascular complications and death occasionally reported (8, 22). Therefore, health care providers should be familiar with the characteristic clinical presentation and pattern of RF and solicit travel histories from patients who present with compatible symptoms. Identification and reporting of RF cases to appropriate public health authorities are critical for appropriate follow-up, as buildings currently or previously infested with rodents can serve as refuges for ticks and a continued source of infection. Recent developments in immunodiagnostics, including Western immunoblotting and GlpQ serology, may provide additional means by which to further define the epidemiologic scope and ecologic distribution of RF.

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REFERENCES