ISOLATION OF BORRELLIA BURGDORFERI FROM NEOTOMA FUSCIPES, PEROMYSCUS MANICULATUS, PEROMYSCUS BOYLII, AND IXODES PACIFICUS IN OREGON

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Abstract. The number of Lyme disease cases in Oregon has increased in recent years despite the fact that the pathogen, Borrelia burgdorferi, has never been isolated in the state. Rodent and tick surveys were undertaken in 1997 to isolate and characterize strains of B. burgdorferi from Oregon and to identify potential reservoirs and vectors of Lyme disease. Borrelia burgdorferi was isolated from Neotoma fuscipes, Peromyscus maniculatus, P. boylii, and Ixodes pacificus. Both N. fuscipes and P. maniculatus were infested with I. pacificus and I. spinipalpis. Although I. pacificus infested P. boylii, I. spinipalpis was not found on this rodent, and only 4% of the P. boylii were infected with B. burgdorferi compared with the 19% and 18% infection rates found in N. fuscipes and P. maniculatus, respectively. Variation in the molecular weights of the outer surface proteins A and B were found in these first confirmed isolates of B. burgdorferi from Oregon, as well as truncated forms of outer surface protein B.

Lyme disease caused by Borrelia burgdorferi is the most common vector-borne disease in the United States. Since the discovery of the etiologic agent in 1981, the known endemic range of Lyme disease together with the numbers of reported cases has steadily increased.¹ The principal vectors of B. burgdorferi are Ixodes scapularis and I. pacificus in the eastern and western United States, respectively.² Recently, I. spinipalpis was found to be a competent Lyme disease vector in enzootic cycles involving mice (Peromyscus spp) and wood rats (Neotoma fuscipes) in California,³ and N. mexicana in Colorado.⁴ Despite reports of I. spinipalpis attaching to humans in Oregon,⁵ New Mexico (Centers for Disease Control and Prevention [CDC], unpublished data), and Canada,⁶ I. spinipalpis is not believed to be important in the transmission of B. burgdorferi to humans since nymphs are usually found either attached to animals or in the more humid environment of a rodent nest.

Reports of Lyme disease cases have been increasing in number and spreading in geographic range in the Pacific Northwest. In Oregon, there were only four reported cases of Lyme disease from two counties in 1991 (CDC, unpublished data) compared with 19 cases from 11 counties reported in 1996.⁷ Although spirochetes were found in almost 2% of I. pacificus in Oregon from 1982 to 1984, these spirochetes were not identified.⁸ The results of a survey undertaken in April–May 1997 to try to confirm the presence of B. burgdorferi in Oregon and to ascertain which animals and ticks may be responsible for the maintenance of this human pathogen in enzootic cycles are presented.

MATERIALS AND METHODS

Field studies. Field studies in southern Oregon were undertaken during 1997 at two sites in Jackson County (Woodrat Mountain and Lost Creek) and one site (Spencer Creek) in Josephine County. Host-seeking adult I. pacificus ticks were collected by flagging vegetation during April 1997 and held at 4°C in a saturated humidity until processed for spirochete isolation in Barbour-Stoenner-Kelly (BSK-H) culture medium (Sigma Chemical Co., St. Louis, MO). In May 1997, the rodent populations at these three sites were surveyed with Sherman (H. B. Sherman Traps, Inc., Tallahassee, FL) and Tomahawk (Tomahawk Live Trap Co., Tomahawk, WI) traps using rolled oats and peanut butter as bait. Traps were checked each morning and captured rodents were processed as described below before being released at their site of capture.

Captured rodents were anaesthetized with Metafane® (Schering-Plough Animal Health Corp., Union, NJ) prior to ectoparasite removal. Rodents were bled and ear biopsy specimens were taken. Blood samples were obtained by cardiac puncture. Blood samples were placed in microtainers and held on ice until centrifuged to separate serum. Ear biopsy specimens were surface-decontaminated by washing in Wescodyne® (Amsco, Erie, PA) for 5 min followed by two 5-min washes in 70% ethanol. Ear biopsy specimens were minced prior to being placed in individual 4-ml, snap-cap containers with 3 ml of BSK-H culture medium supplemented with 6% rabbit serum and antibiotics.⁹¹⁰

Laboratory studies. Ticks were identified using standard taxonomic keys. Larvae were mounted in PVA mounting medium (BioQuip, Gardena, CA) prior to identification.¹¹¹³

Adult I. pacificus ticks were surface-sterilized as described above, crushed, and placed into BSK-H medium. Cultures of both ticks and ear biopsy specimens were examined one, two, and four weeks later for the presence of viable spirochetes at 500× magnification under dark-field microscopy. Two 150-μl samples of spirochete-positive cultures were frozen in 30% sterile glycerol (final concentration). Spirochetes from the remainder of these cultures were washed three times in 0.1 M phosphate-buffered saline with 5 mg/ml of MgCl₂, pH 7.4, and stored at 4°C prior to antigenic analysis.

Protein concentrations of washed spirochetes were determined using the Bio-Rad miniprotein kit (Bio-Rad Laboratories, Hercules, CA). Spirochete antigens were separated under reducing conditions using 10% Tris-glycine polyacrylamide gels (Novex, San Diego, CA). In addition to spirochete antigens from Oregon, molecular weight markers (Bio-Rad prestained low-molecular-weight markers) and B. burgdorferi (B31 strain) antigens were run as controls. Four sets of identical gels were run: one set of gels was silver-stained and the other three were blotted onto nitrocellulose prior to antibody probing. Blots were probed with either a panel of diagnostic anti-B. burgdorferi monoclonal antibodies (MAbs) against the outer surface protein (Osp) A (31 kD),...
OspB (34 kD), OspC, p39, flagellin (Fla) (41 kD), and p93 antigens or with only the anti-OspA MAb or with only anti-OspB MAb.\textsuperscript{14}

**RESULTS**

Viable spirochetes were successfully isolated from 3% of adult *I. pacificus* adults; rodents ear biopsy specimens yielded 15% spirochete-positive cultures (Table 1). Spirochetes were isolated from 19% (15 of 78), 18% (2 of 11), and 4% (1 of 24) of the ear biopsies of *N. fuscipes*, *P. maniculatus*, and *P. boylii*, respectively. Spirochetes were seen in an additional eight BSK-H cultures, but these did not survive. These unknown spirochetes were noted in cultures derived from *N. fuscipes* from the Woodrat Mountain (n = 3), Spencer Creek (n = 2), and Lost Creek (n = 3) study sites.

All silver-stained polyacrylamide gels of spirochete antigens were consistent with an identity of *B. burgdorferi* sensu lato. Antigens from eight spirochete isolates from rodents and eight derived from infected ticks were further characterized by probing Western blots with a panel of MAbs diagnostic for the OspA (MAb H5332), OspB (MAb 84C), OspC (4B8F4), p39 (H1141), fla (MAb H9724), and p93 (MAb 181.1) antigens of *B. burgdorferi* (Figure 1). All spirochete isolates were recognized by the OspA, OspB, p39, fla, and p93 MAbs. The OspC antigen defined by the MAb 4B8F4 was recognized in 14 of the 16 isolates. The amount of OspC expressed also varied. Fourteen of 16 Oregon spirochete isolates had OspA and OspB bands with molecular weights of 32 kD and 33 kD as recognized by the MAbs H5332 and 84C, respectively (Figure 2). Two of the 16 isolates also had truncated forms of OspB. *Ixodes pacificus* larvae were found on all three rodent species collected, with infestation prevalences of 55%, 73%, and 83% for *P. maniculatus*, *N. fuscipes*, and *P. boylii*, respectively (Tables 2 and 3). The mean number of *I. pacificus* larvae on infested rodents was more than twice as great on *N. fuscipes* (6.7) compared with *P. boylii* (3.0) or *P. maniculatus* (3.0) (Table 3). Despite infestation prevalences of 24% (19 of 78) and 36% (4 of 11) on *N. fuscipes* and *P. maniculatus* at the three collection sites, *I. spinipalpis* larvae were not found on *P. boylii* (n = 24). *Ixodes angustus* infested 6% of *N. fuscipes* and 9% of *P. maniculatus*. *Dermacentor occidentalis* nymphs were found on 23% of *N. fuscipes*, 9% of *P. maniculatus*, and 13% of *P. boylii*.

**DISCUSSION**

A number of novel enzootic cycles of Lyme disease have been described from the western United States. In California and Colorado, *B. burgdorferi* is maintained in *Neotoma* spp and *Peromyscus* spp by the vector *I. spinipalpis*.\textsuperscript{3,4} In California, kangaroo rats (*Dipodomys californicus*) also serve as

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**TABLE 1**

<table>
<thead>
<tr>
<th>Collection sites</th>
<th>Woodrat Mountain</th>
<th>Spencer Creek</th>
<th>Lost Creek</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Positive tested (%)</td>
<td>Positive tested (%)</td>
<td>Positive tested (%)</td>
<td>Positive tested (%)</td>
</tr>
<tr>
<td><em>Neotoma fuscipes</em></td>
<td>5/37 (14)</td>
<td>5/24 (21)</td>
<td>5/17 (29)</td>
<td>15/78 (19)</td>
</tr>
<tr>
<td><em>Peromyscus boylii</em></td>
<td>1/23 (4)</td>
<td>0/1 (0)</td>
<td>0/0 –</td>
<td>1/24 (4)</td>
</tr>
<tr>
<td><em>P. maniculatus</em></td>
<td>0/1 (0)</td>
<td>1/8 (12)</td>
<td>1/2 (50)</td>
<td>2/11 (18)</td>
</tr>
<tr>
<td><em>Ixodes pacificus</em></td>
<td>4/67 (6)</td>
<td>0/78 (0)</td>
<td>4/101 (4)</td>
<td>8/246 (3)</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Western blots of 10% polyacrylamide gels containing spirochete isolates from southern Oregon. Spirochetes in the Ticks gel were isolated from *Ixodes pacificus* adults (lanes 3–10) while spirochetes in the Rodents gel were isolated from *Neotoma fuscipes* (lanes 3, 4, 6, 7, 8, 9, and 10), and *Peromyscus boylii* (lane 5). Lanes 1 and 2 contain prestained molecular weight markers (Bio-Rad Laboratories) and *Borrelia burgdorferi* B31 antigens as controls, respectively. Blots were probed with monoclonal antibodies against the outer surface proteins (Osp)A, B, and C, as well as the flagellin (Fla), p39, and p93 antigens.
a reservoir for *B. burgdorferi* with *I. pacificus* also serving as a vector.3,15

In this survey, spirochetes were isolated from both rodents and ticks and identified as *B. burgdorferi* by diagnostic MAb recognition. Like the *B. burgdorferi* strains in California,16 variability in the molecular weights of OspA and OspB from Oregon were noted, with most Oregon isolates having OspA and OspB molecular weights of 32 kD and 33 kD, respectively. As previously noted in California,16 several of the Oregon *B. burgdorferi* isolates had truncated forms of OspB. Variation in antibody reactivity to OspCs has been previously reported17,18 and undoubtedly explains our results in which only 14 of 16 *B. burgdorferi* isolates from Oregon were recognized by the anti-OspC MAb. Like the California isolates of *B. burgdorferi*,16 variability in the amount of OspC expressed among the Oregon isolates was noted. Unusual *B. burgdorferi* isolates, such as DN-127, which lack OspA and OspB but possess a major 25-kD protein,19,20 constitute as many as 9% of the strains in California.3 However, in this survey, none of the isolates was of the DN-127 type.

Aspects of the enzootic Lyme disease cycles seen in California were found in Oregon. *Borrelia burgdorferi* was isolated from the reservoir hosts *N. fascipes*, *P. maniculatus*, and *P. boylii*. *Ixodes pacificus* was identified as a potential vector. Interestingly, we isolated *B. burgdorferi* from only 4% of *P. boylii*. In contrast, 22% of *P. boylii* in California were infected with *B. burgdorferi*.21 The absence of *I. spinipalpis* on *P. boylii*, together with the low *B. burgdorferi* infection rate in *P. boylii*, suggests that *I. spinipalpis* is important in maintaining *B. burgdorferi* in enzootic cycles in the Pacific Northwest. Evidence in support of this hypothesis is seen in the prevalence of *I. pacificus* larvae on *P. boylii*, which was greater than on *P. maniculatus*, yet *P. maniculatus* had a higher *B. burgdorferi* infection rate. Although this study did not attempt to isolate spirochetes from *I. spinipalpis*, it is probable that this tick is important in the maintenance of Lyme disease in the Pacific Northwest because it is known to be a permissive vector of Lyme disease22 and it was collected on confirmed reservoir species. Additional studies with larger sample sizes will be required to confirm whether these observations are statistically and biologically significant.

Since *I. spinipalpis* often has much higher *B. burgdorferi* infection rates than *I. pacificus*, it potentially can pose a much greater threat of transmission to humans. Because of the low humidity found in much of its range, this species is predominantly nidiculous, either remaining on the rodent or in the rodent’s nest. However, it has been reported attached to humans in New Mexico (CDC, unpublished data), Canada,8 and in Linn County, Oregon,3 indicating that it may quest for hosts outside rodent nests under the right environmental conditions. In fact, Gregson20 reported that *I. spinipalpis* has been collected off clothing as well as on birds and by dragging. It may be that such questing behavior is not uncommon in the more humid conditions of the Pacific Northwest. Questing behavior outside rodent nests together with a potentially high *B. burgdorferi* infection rate in *I. spinipalpis* would pose an increased risk to humans for *B. burgdorferi* transmission in the northwestern United States.

**Table 2**

<table>
<thead>
<tr>
<th>Species</th>
<th>Number, stage* and species of ticks</th>
<th>Rodents</th>
<th>Isodex pacificus</th>
<th>I. spinipalpis</th>
<th>I. angustus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td></td>
<td>Species</td>
<td>No. N L A N L A</td>
<td>N L A N L A</td>
<td>N L A N L A</td>
</tr>
<tr>
<td>Woodrat Mountain</td>
<td><em>Neotoma fascipes</em></td>
<td>37</td>
<td>0 302 0 2 1 5 0</td>
<td>0 4 0 0 0 0 0</td>
<td>0 3 0 0 0 0 0</td>
</tr>
<tr>
<td>Jackson County</td>
<td><em>Peromyscus boylii</em></td>
<td>23</td>
<td>0 59 0 0 0 0 0</td>
<td>0 5 0 0 0 0 0</td>
<td>0 5 0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td><em>P. maniculatus</em></td>
<td>1</td>
<td>0 1 0 0 0 0 0 0</td>
<td>0 1 0 0 0 0 0</td>
<td>0 1 0 0 0 0 0</td>
</tr>
<tr>
<td>Spencer Creek</td>
<td><em>N. fascipes</em></td>
<td>24</td>
<td>1 39 1 29 36 0</td>
<td>2 37 0 0 0 0 0</td>
<td>2 37 0 0 0 0 0</td>
</tr>
<tr>
<td>Josephine County</td>
<td><em>P. boylii</em></td>
<td>1</td>
<td>0 1 0 0 0 0 0 0</td>
<td>0 1 0 0 0 0 0</td>
<td>0 1 0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td><em>P. maniculatus</em></td>
<td>8</td>
<td>0 4 0 5 0 0 0 0</td>
<td>0 1 0 0 0 0 0</td>
<td>0 1 0 0 0 0 0</td>
</tr>
<tr>
<td>Lost Creek Reservoir,</td>
<td><em>N. fascipes</em></td>
<td>17</td>
<td>2 43 1 9 20 0 0 0</td>
<td>0 2 0 0 0 0 0</td>
<td>0 2 0 0 0 0 0</td>
</tr>
<tr>
<td>Jackson County</td>
<td><em>P. maniculatus</em></td>
<td>2</td>
<td>0 7 0 1 0 0 0 0</td>
<td>0 2 0 0 0 0 0</td>
<td>0 2 0 0 0 0 0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>113</td>
<td>3 456 2 40 63 5</td>
<td>0 5 1 0 0 0 0</td>
<td>0 5 1 0 0 0 0</td>
</tr>
</tbody>
</table>

* N = nymph, L = larva, A = adult.
Finally, the importance of _I. pacificus_ as a vector of Lyme disease in the Pacific Northwest should not be underestimated. In much of its range, _I. pacificus_ coexists with the western fence lizard, _Sceloporus occidentalis_, a preferred host of immature forms of _I. pacificus_ in California. The blood of _S. occidentalis_ contains a thermostable borrelia-cidal factor that destroys _B. burgdorferi_ midgut diventricula of _I. pacificus_, thereby lessening the Lyme disease transmission potential of this tick. However, the distribution of _I. pacificus_ extends farther north into parts of Washington State and Canada, where the western fence lizard is not found. In such areas, one would predict greater infestation rates of _I. pacificus_ on rodents in much the same way that northern populations of _I. scapularis_ feed predominantly on rodents while more southern populations predominately feed on lizards. In these more northern parts of the range of _I. pacificus_, higher _B. burgdorferi_ infection rates might be expected with concurrent increased risk of Lyme disease transmission to humans.

This preliminary study on the transmission and maintenance of _B. burgdorferi_ in Oregon has generated a number of observations that will require further research. These include the relative importance of _I. spinipalpis_ and _I. pacificus_ in the maintenance of _B. burgdorferi_ infections in rodents in the Pacific Northwest as well as the possibility of transmission of _B. burgdorferi_ to humans by questing _I. spinipalpis_. Finally, questions regarding the relationship of _I. pacificus_ density on rodents and infection with _B. burgdorferi_ where the western fence lizard is not found deserve additional study.

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### REFERENCES

15. Lane RS, Brown RN, 1991. Wood rats and kangaroo rats: po-


