

## ISOLATION OF *BORRELIA BURGdorFERI* FROM *NEOTOMA FUSCIPES*, *PEROMYSCUS MANICULATUS*, *PEROMYSCUS BOYLI*, 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 infested 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.<sup>1</sup> The principal vectors of *B. burgdorferi* are *Ixodes scapularis* and *I. pacificus* in the eastern and western United States, respectively.<sup>2</sup> 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,<sup>3</sup> and *N. mexicana* in Colorado.<sup>4</sup> Despite reports of *I. spinipalpis* attaching to humans in Oregon,<sup>5</sup> New Mexico (Centers for Disease Control and Prevention [CDC], unpublished data), and Canada,<sup>6</sup> *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.<sup>7</sup> Although spirochetes were found in almost 2% of *I. pacificus* in Oregon from 1982 to 1984, these spirochetes were not identified.<sup>8</sup> 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., Toma-

hawk, 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.<sup>9,10</sup>

**Laboratory studies.** Ticks were identified using standard taxonomic keys. Larvae were mounted in PVA mounting medium (BioQuip, Gardena, CA) prior to identification.<sup>11–13</sup>

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 MgCl<sub>2</sub>, pH 7.4, and stored at 4°C prior to antigenic analysis.

Protein concentrations of washed spirochetes were determined using the Bio-Rad mini-protein 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),

TABLE 1  
Spirochetes isolated in Barbour-Stoenner-Kelly-H media from southern Oregon

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

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.<sup>14</sup>

#### 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*.<sup>3,4</sup> In California, kangaroo rats (*Dipodomys californicus*) also serve as

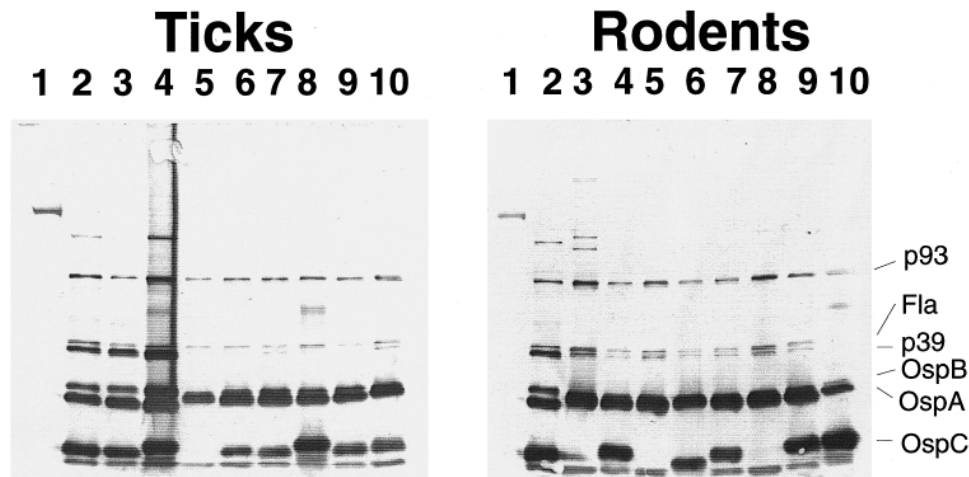


FIGURE 1. Western blots of 10% polyacrylamide gels containing spirochete isolates from southern Oregon. Spirochetes in the Ticks gel were isolated from *Ixodes pacificus* adults (lanes 3–10) while spirochetes in the Rodents gel were isolated from *Neotoma fuscipes* (lanes 3, 4, 6, 7, 8, 9, and 10), and *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.

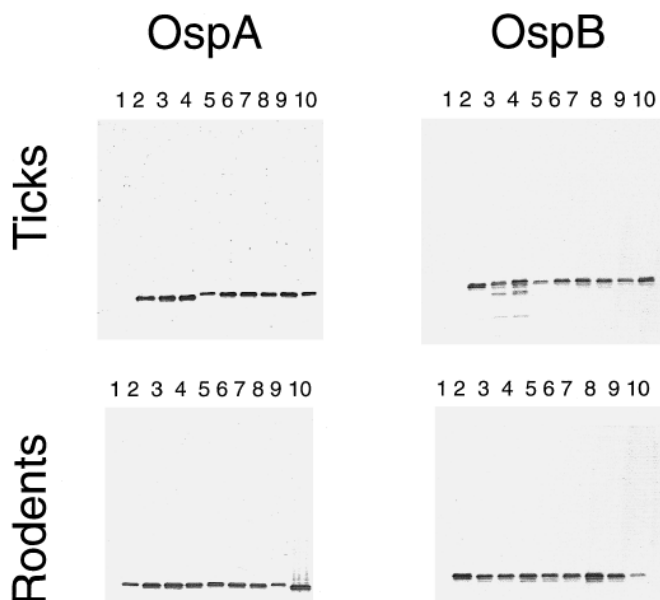


FIGURE 2. Western blot analyses of *Borrelia burgdorferi* antigens from Oregon isolated from *Ixodes pacificus* adults (lanes 3–10; Ticks gels) and rodents (spirochete antigens isolated from *Neotoma fuscipes* are shown in lanes 3, 4, 6, 7, 8, 9, and 10; those from *Peromyscus boylii* are shown in lane 5). Blots were probed with monoclonal antibodies recognizing the outer surface protein (Osp)A or OspB. Lanes 1 and 2 contain molecular weight markers and *Borrelia burgdorferi* strain 31 antigens, respectively. Note the truncated forms of OspB seen in spirochetes isolated from the ticks in lanes 3 and 4.

a reservoir for *B. burgdorferi* with *I. pacificus* also serving as a vector.<sup>3,15</sup>

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,<sup>16</sup> 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,<sup>16</sup> several of the Oregon *B. burgdorferi* isolates had truncated forms of OspB. Variation in antibody reactivity to OspCs has been previously reported<sup>17,18</sup> 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*,<sup>16</sup> 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,<sup>19,20</sup> constitute as many as 9% of the strains in California.<sup>3</sup> 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. fuscipes*, *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*.<sup>21</sup> 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 known to be a permissive vector of Lyme disease<sup>22</sup> 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 nidicolous, 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,<sup>6</sup> and in Linn County, Oregon,<sup>5</sup> indicating that it may quest for hosts outside rodent nests under the right environmental conditions. In fact, Gregson<sup>6</sup> 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  
Ticks collected from rodents in southern Oregon in May 1997

Location	Rodents Species	No.	Number, stage,* and species of ticks							Derma- centor occidentalis N
			<i>Ixodes pacificus</i>			<i>I. spinipalpis</i>		<i>I. angustus</i>		
			N	L	A	N	L	N	L	
Woodrat Mountain Jackson County	<i>Neotoma fuscipes</i>	37	0	302	0	2	1	5	0	14
	<i>Peromyscus boylii</i>	23	0	59	0	0	0	0	0	2
	<i>P. maniculatus</i>	1	0	1	0	0	0	0	0	0
Spencer Creek Josephine County	<i>N. fuscipes</i>	24	1	39	1	29	36	0	0	37
	<i>P. boylii</i>	1	0	1	0	0	0	0	0	2
	<i>P. maniculatus</i>	8	0	4	0	0	5	0	1	1
Lost Creek Reservoir, Jackson County	<i>N. fuscipes</i>	17	2	43	1	9	20	0	0	2
	<i>P. maniculatus</i>	2	0	7	0	0	1	0	0	0
	Total	113	3	456	2	40	63	5	1	58

\* N = nymph; L = larva; A = adult.

TABLE 3  
Prevalence and mean number of ticks on infested rodents in southern Oregon

Host species	Tick species	Larvae		Nymphs		Adults	
		Prevalence (%)	Mean*	Prevalence (%)	Mean*	Prevalence (%)	Mean*
<i>Neotoma fuscipes</i>	<i>Ixodes pacificus</i>	73	6.7	5	1.0	0	—
	<i>I. spinipalpis</i>	24	2.8	28	1.8	3	1.0
	<i>I. angustus</i>	0	—	6	1.0	0	—
	<i>Dermacentor occidentalis</i>	0	—	23	2.9	0	—
<i>Peromyscus maniculatus</i>	<i>I. pacificus</i>	55	3.0	0	—	0	—
	<i>I. spinipalpis</i>	36	2.0	0	—	0	—
	<i>I. angustus</i>	9	1.0	0	—	0	—
	<i>D. occidentalis</i>	0	—	9	1.0	0	—
<i>P. boylii</i>	<i>I. pacificus</i>	83	3.0	0	—	0	—
	<i>D. occidentalis</i>	0	—	13	1.3	0	—

\* Mean represents the mean number of ticks on rodents with one or more ticks (e.g., the number of ticks on infested rodents only).

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.<sup>23</sup> The blood of *S. occidentalis* contains a thermostabile borreliacidal factor that destroys *B. burgdorferi* spirochetes in the midgut diverticula of *I. pacificus* nymphs, thereby lessening the Lyme disease transmission potential of this tick.<sup>24</sup> However, the distribution of *I. pacificus* extends farther north into parts of Washington State<sup>2</sup> and Canada<sup>6</sup>, where the western fence lizard is not found.<sup>25</sup> 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 predominantly feed on lizards.<sup>26</sup> 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 deserves additional study.

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