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Authors: Lane, Robert S., and Burgdorfer, Willy

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SPIROCHETES IN MAMMALS AND TICKS (ACARI: IXODIDAE) FROM A FOCUS OF LYME BORRELIOSIS IN CALIFORNIA

Robert S. Lane¹ and Willy Burgdorfer²

¹ Department of Entomological Sciences, University of California, Berkeley, California 94720, USA

² Laboratory of Pathobiology, Rocky Mountain Laboratories, NIAID, Hamilton, Montana 59840, USA

ABSTRACT: In northern California, antibodies to *Borrelia burgdorferi* were detected in 58 of 73 (79%), and spirochetemias in one of 26 (4%) black-tailed jackrabbits (*Lepus californicus californicus*), by indirect and direct immunofluorescence, respectively. Five species of ticks (*Dermacentor occidentalis*, *D. parumapertus*, *Ixodes neotomae*, *I. pacificus*, and *Haemaphysalis leporispalustris*) were collected from rabbits. Two of these species of ticks were found to contain spirochetes; two of 10 (20%) *I. neotomae* and two of 174 (1%) *H. leporispalustris*. A strain of *B. burgdorferi* was recovered from *I. neotomae*. One infected *H. leporispalustris* female passed spirochetes via eggs to about 67% of her progeny. The widespread distribution of the black-tailed jackrabbit, its infestation by at least four ticks (*D. occidentalis*, *D. parumapertus*, *I. neotomae*, and *I. pacificus*) known to be infected naturally with *B. burgdorferi*, and the high prevalence of spirochetal antibody in this lagomorph suggest that it might be useful as a sentinel for surveillance of Lyme borreliosis. Spirochetes were detected in 15% of 40 Columbian black-tailed deer (*Odocoileus hemionus columbianus*) by direct immunofluorescence bound with a *Borrelia*-specific monoclonal antibody (H9724), but not with a monoclonal antibody (H5332) specific for *B. burgdorferi*. The geographical overlap of different borreliæ in ticks that bite wildlife such as deer may confound spirochetal serosurveys, and underscores the need for more specific serologic tests than those currently available.

Key words: Lyme borreliosis, spirochetes, ticks, jackrabbits, deer, natural infections, *Borrelia burgdorferi*.

INTRODUCTION

In the far western United States, the spirochete causing Lyme borreliosis (*Borrelia burgdorferi*) has been isolated from the western black-legged tick (*Ixodes pacificus*) and antibodies to it or to related spirochetes have been detected in Columbian black-tailed deer (*Odocoileus hemionus columbianus*), axis deer (*Cervus axis*), fallow deer (*Cervus dama*), and humans (Campagna et al., 1983; Burgdorfer et al., 1985; Lane and Burgdorfer, 1986).

Lagomorphs have been implicated as hosts of *B. burgdorferi* once previously. Two of 10 jackrabbits from West Texas yielded antibodies to *B. burgdorferi* at titers of 1:64 (Rawlings, 1986). Since the black-tailed jackrabbit (*Lepus californicus californicus*) is a significant host of *I. pacificus* in northern California (Lane et al., 1981), a study was undertaken to evaluate the role of this lagomorph and its ticks in the ecology of *B. burgdorferi* at a well-established focus of Lyme borreliosis in Mendocino County (Lane and Burgdorfer, 1986). We present the results of this study

and provide evidence that in the same region Columbian black-tailed deer serve as hosts of a spirochete resembling the recently-described *Borrelia coriaceae*, which is transmitted by the soft tick *Ornithodoros coriaceus* (Lane et al., 1985; Johnson et al., 1987). The implication of the latter host-parasite association for interpretation of deer serologic data in areas where *B. burgdorferi* and *B. coriaceae* overlap is discussed.

MATERIALS AND METHODS

Black-tailed jackrabbits and Columbian black-tailed deer were collected at the University of California, Hopland Field Station in southeastern Mendocino County (39°00'N, 123°04'W). Descriptions of the study area and its climate have been given previously (Heady, 1961; Pitt and Heady, 1978).

Rabbits and deer were collected by shooting; the former in 1976, 1977, 1985 and 1986, the latter only in August of 1985 and 1986. Sera were obtained from rabbits taken in all years, and multiple thick blood films were prepared from each rabbit and deer collected in 1985 and 1986. In addition, the entire body of each rabbit was inspected for approximately 10-15 min for ticks, and specimens found were identified and



TABLE 1. Prevalence of antibodies to *Borrelia burgdorferi* in black-tailed jackrabbits from the University of California Hopland Field Station.

Year	Number of sera tested	Number (%) positive	Reciprocal titration end points ^a			
			64	128	256	≥512
1976	16	12 (75)	4	4	2	2
1977	33	28 (85)	7	5	7	9
1985	4	4 (100)	0	2	1	1
1986	20	14 (70)	2	8	2	2
Totals	73	58 (79)	13	19	12	14

^a Indirect immunofluorescence test.

survivors were examined for spirochetes (see below).

Rabbit sera were assayed for antibodies to *B. burgdorferi* strain B-31 by indirect immunofluorescence (Russell et al., 1984) with a fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit conjugate diluted 1:800 (Antibodies Incorporated, Davis, California 95617, USA). Experimentally prepared hyperimmune domestic rabbit serum and normal domestic rabbit serum were used as positive and negative controls, respectively. A minimum reciprocal titer of 64 was considered evidence of previous spirochetal exposure.

One of three thick blood films prepared from each of 40 deer and 26 rabbits was examined for spirochetes by direct immunofluorescence (DI) with a polyvalent conjugate against *B. burgdorferi* prepared in rabbits (Lane and Burgdorfer, 1986). If spirochetes were not detected, a second blood film was examined similarly. If spirochetes were observed, an attempt was made to identify them on the remaining blood films with monoclonal antibodies, as described below. Also, in attempts to isolate spirochetes, ≤0.1 ml of whole blood obtained by cardiac puncture from 18 deer and 20 rabbits were inoculated into 6–9 ml of BSK II medium (Barbour, 1984) with or without rifampin (50 µg/ml). The culture tubes were maintained at 34–35 C and checked for spirochetal growth at weekly intervals for 1 mo.

Surviving ticks, following identification, were inspected for spirochetes by DI examination of whole body smears (larval and nymphal ticks) or individually-dissected tissues (adult ticks) as described previously (Lane et al., 1985; Lane and Burgdorfer, 1986). Twenty-eight engorged females of the rabbit tick, *Haemaphysalis leporispalustris*, were held for oviposition in a desiccator at a relative humidity of approximately 85%. After ovipositing, surviving *H. leporispalustris* females were dissected and smears of various tissues including midgut, central ganglion, and ovary were tested for spirochetes by

DI. Whole body smears of 52 F₁ larvae from one infected female were tested likewise for spirochetes. Also, tissue smears from spirochete-infected ticks were fixed in absolute methanol (3 min) and then stained with Giemsa (60 min).

Spirochetes detected in smears of tick tissues or in deer blood films by DI were identified to genus or species by indirect immunofluorescence with murine monoclonal antibodies H9724 and H5332, respectively (courtesy of Dr. Alan Barbour); preparation H9724 binds only with members of the genus *Borrelia*, whereas H5332 is specific for *B. burgdorferi* (Barbour et al., 1983, 1986).

Seropositivity rates in rabbits were not compared between years inasmuch as there was significant seasonal variation in collections. In deer, the prevalence of spirochetemia by year was compared with Fisher's exact test (2-tailed) (Zar, 1974). A 5% level of probability was set for rejecting the null hypothesis.

RESULTS

In 1976 and 1977, antibodies to *B. burgdorferi* at reciprocal titers ranging from 64 to ≥512 were detected in 75% and 85% of the rabbits, respectively (Table 1). Similarly, 100% of the few rabbits collected in 1985 were seropositive as were 70% of those taken in 1986. Overall, seropositive rabbits were collected in all seasons as follows: winter (3 of 4 animals), spring (34 of 39), summer (8 of 13), and fall (13 of 17). Seropositivity rates were similar in males versus females and in young-of-year versus adults.

The DI examination of thick blood films revealed the presence of spirochetes in one (14%) of seven rabbits collected in spring. The spirochetes stained brightly with the polyvalent conjugate and were similar

morphologically to *B. burgdorferi* observed previously in midgut tissues of *I. pacificus* (Burgdorfer et al., 1985; see fig. 2A). In the indirect immunofluorescence test, however, these spirochetes did not bind with *B. burgdorferi*-specific monoclonal antibodies (H5332). The spirochetemic rabbit also had an antibody titer of 1:128 against *B. burgdorferi*. None of the rabbits obtained in other seasons (winter, 2; summer, 8; fall, 9) was spirochetemic.

We failed to isolate spirochetes from blood samples of 20 rabbits collected in May ($n = 4$), July ($n = 6$), September ($n = 6$), or December ($n = 4$). Also, spirochetes were not detected in duplicate thick blood films prepared from each of these animals.

Spirochetemias were absent in 15 and present in six of 25 (24%) deer examined in 1985 and 1986, respectively. Year-to-year variation in the prevalence of spirochetemias did not differ significantly ($P = 0.067$). In thick blood films, spirochetes ranged in number from two to 20 organisms per specimen, reacted moderately well with the polyvalent conjugate, and resembled *Borrelia coriaceae* morphologically and immunologically. Further, they bound with the *Borrelia*-specific monoclonal antibody H9724, but not with the *B. burgdorferi*-specific H5332. Attempts to culture spirochetes from 18 deer blood samples in 1986, which included four of the six that were shown to be spirochetemic by DI, were unsuccessful.

The prevalence of ixodid ticks on rabbits varied seasonally except for *H. leporispalustris*, which was found throughout the year (Table 2). *Haemaphysalis leporispalustris* was the most abundant tick on rabbits in spring and fall (Table 2), while larvae of *Dermacentor* sp. and nymphs of *D. occidentalis* predominated in summer. Low numbers of *H. leporispalustris*, *I. neotomae*, and *I. pacificus* were found on the few animals taken in fall and winter. *Dermacentor parumapertus* appeared to be uncommon in the Hopland area.

Many ticks died before they could be

tested, particularly larvae of *Dermacentor* sp. and males of *H. leporispalustris*. Nevertheless, of the surviving ticks, two of 174 (>1%) *H. leporispalustris* (all stages) and two of 10 (20%) adult *I. neotomae* harbored spirochetes (Table 3). All four positive ticks were engorged females, and at least three of them had generalized spirochetal-infections involving most or all of their tissues (Table 4). The midguts of all four infected ticks contained spirochetes and were heavily infected in three of them.

Spirochetes in tissue smears of both positive *I. neotomae* were identified as *B. burgdorferi* by their immunoreactivity with the specific monoclonal antibody H5332, and those in one of the ticks were isolated in BSK II medium. Spirochetes present in tissues of one of the two positive *H. leporispalustris* females (specimen 4-4) reacted weakly with the anti-*B. burgdorferi* polyvalent conjugate and stained well with Giemsa, but inadvertently none of the spirochetes observed in tissue smears of either positive *H. leporispalustris* female was tested with monoclonal antibodies.

Before dissection, *H. leporispalustris* female 4-4 laid 143 eggs, 136 (95%) of which hatched. Whole body smears of 35 of 52 (67%) F_1 larvae examined by DI contained spirochetes that reacted weakly with the polyvalent conjugate like those visualized in tissues of the parental female. Spirochetes present in tissue smears of F_1 filial ticks reacted to monoclonal antibody H9724, but not to H5332.

DISCUSSION

In North America, *B. burgdorferi* or similar spirochetes have been recovered from or detected in several species of vertebrates besides humans including seven species of birds in the order Passeriformes and seven species of mammals in the orders Artiodactyla, Carnivora, and Rodentia (Anderson et al., 1983, 1985, 1986; Bosler et al., 1983, 1984; Anderson and Magnarelli, 1984; Lissman et al., 1984; Levine et al., 1985; Loken et al., 1985). Of these, white-footed mice (*Peromyscus leu-*

TABLE 2. Prevalence and abundance of ixodid ticks by life stage^a on black-tailed jackrabbits from the University of California Hopland Field Station.

Date	Number of rabbits	<i>Dermacentor</i> sp.	Prevalence ^b (mean number of ticks, range) [life stage]			
			<i>Dermacentor occidentalis</i>	<i>Dermacentor parumapertus</i>	<i>Haemaphysalis leporispalustris</i>	<i>Ixodes pacificus</i>
Spring 1985	3	0	0	0	100 (19.0, 12-32) [1 N, 33 ♂, 23 ♀]	0
Fall 1985	1	0	0	0	100 (NA) ^c	100 (NA)
Winter 1986	2	0	0	0	[2 N, 1 ♂, 1 ♀] 100 (1.5, 1-2)	[1 ♀] 100 (3.5, 1-6)
Spring 1986	4	0	0	25 (0.5, 0-2) [2 ♀]	[1 N, 2 ♀] 100 (61.0, 48-74) [1 N, 142 ♂, 101 ♀]	[4 ♂, 3 ♀] 50 (1.3, 0-3) [2 N, 1 ♂, 2 ♀]
Summer 1986	8	88 (59.5, 0-164) [476 L]	100 (15.1, 1-38) [28 L, 93 N] ^d	0	100 (12.9, 1-47) [4 L, 4 N, 78 ♂, 17 ♀]	0
Fall 1986	8	0	13 (0.1, 0-1) [1 N]	0	75 (3.6, 0-10) [14 L, 11 N, 3 ♂, 1 ♀]	25 (0.8, 0-4) [2 ♂, 4 ♀]

^a L, larva; N, nymph.^b Percentages of rabbits that were tick-infested.^c Mean and range not applicable since only one animal was examined.^d Twenty-eight replete larvae were determined to species after they had molted to nymphs.

TABLE 3. Prevalence of spirochetes in ixodid ticks collected from black-tailed jackrabbits at the University of California Hopland Field Station.

Date	Number of ticks positive/number examined (life stage) ^a					
	<i>Dermacen- tor</i> sp.	<i>Derma- centor occiden- talis</i>	<i>Derma- centor paruma- pertus</i>	<i>Haemaphysalis leporispalustris</i>	<i>Ixodes neotomae</i>	<i>Ixodes pacificus</i>
Spring 1985	—	—	—	1/42 (1 N, 20 ♂, 21 ♀)	—	—
Fall 1985	—	—	—	0/4 (2 N, 1 ♂, 1 ♀)	2/2 ♀	0/1 ♀
Winter 1986	—	—	—	0/3 (1 N, 2 ♀)	0/3 (2 ♂, 1 ♀)	0/7 (4 ♂, 3 ♀)
Spring 1986	—	—	0/1	1/70 (1 N, 1 ♂, 68 ♀)	—	0/1 N
Summer 1986	0/10 L	0/104 N	—	0/33 (2 L, 6 N, 7 ♂, 18 ♀)	—	—
Fall 1986	—	0/1 N	—	0/22 (8 L, 11 N, 2 ♂, 1 ♀)	0/5 (1 ♂, 4 ♀)	0/4 (2 ♂, 2 ♀)
Totals	0/10 L	0/105 N	0/1	2/174 (10 L, 22 N, 31 ♂, 111 ♀)	2/10 (3 ♂, 7 ♀)	0/13 (1 N, 6 ♂, 6 ♀)

^a L, larva; N, nymph.

copus) and white-tailed deer (*Odocoileus virginianus*) have been implicated as reservoirs of the spirochete (Bosler et al., 1983; Levine et al., 1985; Donahue et al., 1987). We have demonstrated that a lagomorph, the black-tailed jackrabbit, is involved in the epizootiology of *B. burgdorferi* or related spirochetes in the far western United States.

In California, the black-tailed jackrabbit inhabits nearly every terrestrial community except those in the Sonoran and Transition life zones of the higher mountains (Ingles, 1965). Its distribution coincides with that of the western black-legged tick (*I. pacificus*), the primary vector of Lyme borreliosis in this state. This species of tick has been recorded from 50 of 58 counties from sea level to elevations >2,150 m (Furman and Loomis, 1984). Thus, the black-tailed jackrabbit may be useful as a sentinel animal for surveillance of Lyme borreliosis in California since it is widely distributed in a variety of habitats, is capable of breeding throughout the year (Ingles, 1965), serves as a host of four species of ticks (*D. occidentalis*, *D. parumapertus*,

I. neotomae, and *I. pacificus*) found to harbor *B. burgdorferi* (Burgdorfer et al., 1985; Rawlings, 1986; present study; R. S. Lane, unpubl. data) and yielded a high seropositivity rate in the present study. Additionally, another lagomorph (*Oryctolagus cuniculus*) has been shown experimentally to be susceptible to *B. burgdorferi* (Benach et al., 1984; Burgdorfer, 1984; Kornblatt et al., 1984a, b).

A possible caveat in using jackrabbits as a sentinel for *B. burgdorferi*, however, is the occurrence in the rabbit tick (*H. leporispalustris*) of an unidentified species of *Borrelia*. This spirochete exhibited reduced immunoreactivity to an anti-*B. burgdorferi* polyvalent conjugate in one female tick and its F₁ (larval) progeny. Moreover, borreliae in filial ticks did not bind with monoclonal antibodies specific for *B. burgdorferi*, and they appeared to stain more deeply with Giemsa than does *B. burgdorferi*. On the other hand, *B. burgdorferi* passed via eggs and from stage-to-stage in *I. pacificus* displays reduced immunofluorescence staining reactivity to polyvalent conjugates and also does not

TABLE 4. Distribution and relative abundance of spirochetes in ixodid ticks collected from black-tailed jackrabbits, University of California Hopland Field Station, 1985–1986, as determined by direct immunofluorescence.

Tick species	Date of collection	Spirochetal infection ^a				
		Central ganglion	Malpighian tubule	Midgut	Ovary	Salivary gland
<i>Haemaphysalis</i>	20 May 1985	—	NT ^b	+++	NT	NT
<i>leporispalustris</i>	9 May 1986 ^c	+++	—	+	+++	—
<i>Ixodes neotomae</i>	19 December 1985	++	++	+++	++	++
	19 December 1985	+++	+	+++	++	++

^a +, <1 spirochete; ++, 1–10 spirochetes; and +++, >10 spirochetes/400×, on average, per several fields; —, devoid of spirochetes.

^b Not tested.

^c The specimen (4-4) collected on this date passed spirochaetes through eggs to most of her progeny (see text).

bind consistently with specific monoclonal antibodies (Lane and Burgdorfer, 1987). We were unsuccessful in isolating the *H. leporispalustris* spirochete and therefore were unable to identify it by other techniques. If this spirochete should prove to be an undescribed species of *Borrelia*, then jackrabbits exposed to it would have cross-reactive antibody against the *B. burgdorferi* antigen used in our indirect immunofluorescence test.

In Connecticut, spirochetes of undetermined species were detected in the midgut of a nymphal *H. leporispalustris* that had been removed from a Swainson's thrush (*Hylocichla ustulata*) (Anderson and Magnarelli, 1984), but transovarial passage of spirochetes in this tick species has not been demonstrated prior to our study. Transmission of *B. burgdorferi* via eggs, however, has been shown to occur in four members of the "*Ixodes ricinus* complex," *I. dammini*, *I. pacificus*, *I. ricinus*, and *I. scapularis* (Burgdorfer et al., 1983; Magnarelli et al., 1986, 1987; Piesman et al., 1986; Lane and Burgdorfer, 1987).

Haemaphysalis leporispalustris is a three-host tick recorded from Alaska, Canada, all of the United States, Mexico, and south to Argentina (Cooley, 1946). It is a common parasite of lagomorphs (*Lepus* spp. and *Sylvilagus* spp.), and is found also on birds and other mammals, particularly rodents. In California, it has been recorded from 12 avian and 16 mammalian species

in 35 of 58 counties (Furman and Loomis, 1984). It is insignificant as a vector of pathogens to humans since it bites people rarely (Brown, 1945), but it is considered an ideal vector of pathogens transmitted between birds and mammals (Jellison, 1974). If the spirochete detected in *H. leporispalustris* in California and Connecticut should prove to be *B. burgdorferi* then the rabbit tick, because of its abundance and widespread distribution, may be important in perpetuating this agent in enzootic foci.

The prevalence of *H. leporispalustris* on black-tailed jackrabbits in the present study (92%, $n = 26$) is similar to that reported for the same tick in an earlier investigation at the Hopland Field Station in which 87% of 107 rabbits were infested (Lane et al., 1981). Elsewhere in California, and in accord with our findings, *H. leporispalustris* was found on *L. californicus* throughout the year in Butte County, with the majority of ticks attached on the head and ears (Lechleitner, 1959). In Monterey County, all 10 jackrabbits examined were parasitized by adult *H. leporispalustris* ($\bar{x} = 8.7$ ticks) and 50% of them were parasitized by immature *H. leporispalustris* ($\bar{x} = 3.2$) as well (Coultrip et al., 1973). Our abundance data for all stages of this tick combined ranged from mean values of 1.5 to 61.0 in different seasons, although these data and those for the other tick species should be considered underestimates since

our collection method (hand picking with forceps) is not very efficient (Westrom, 1975; Westrom et al., 1985).

Ixodes (Ixodes) neotomae has not been implicated previously in the epizootiology of Lyme borreliosis. In California, it occurs commonly on woodrats (*Neotoma* spp.) and lagomorphs (*L. californicus* and *Sylvilagus* spp.), and infrequently on deer mice (*Peromyscus* sp.) and gray fox (*Urocyon cinereoargenteus*) (Furman and Loomis, 1984). To our knowledge, it has not been reported to attack humans. Its known geographical distribution includes California and New Mexico (Keirans and Clifford, 1978). That two of 10 *I. neotomae* examined in the present study contained *B. burgdorferi* suggests that this tick may be involved in transmitting spirochetes among populations of jackrabbits and woodrats.

The similarity of spirochetes detected in blood samples of black-tailed deer in August and those visualized in tissue smears of *O. coriaceus* ticks provides the first evidence that deer may serve as a natural host of the recently-described *B. coriaceae* (Lane et al., 1985; Johnson et al., 1987). If so, then the *O. coriaceus*/*B. coriaceae*/deer association would be unique because the main sources of spirochetal infections for other *Ornithodoros* spp. are rodents and lagomorphs except for *O. moubata* (Burgdorfer, 1985). The latter tick acquires *Borrelia duttonii* while feeding on humans, the only known vertebrate reservoir of this spirochete.

Persistence of *B. coriaceae* in the foci of infection presumably occurs as a result of its prolonged survival within tick tissues after uninfected ticks have become infected while feeding on spirochetemic deer. *Borrelia coriaceae* is occasionally passed via eggs by ticks (Lane et al., 1985), but recent experimental evidence suggests that transovarial passage is inefficient for maintaining this spirochete in populations of *O. coriaceus* (R. S. Lane and S. A. Manweiler, unpubl. data).

Serosurveys of deer for *B. burgdorferi*

in California, like those involving jackrabbits, may be confounded by the occurrence of at least two borreliae in the ticks feeding on deer. At the Hopland Field Station, *B. burgdorferi* and *B. coriaceae* have been recovered from *I. pacificus* and *O. coriaceus*, respectively (Burgdorfer et al., 1985; Lane et al., 1985). Recently, we suggested that the high prevalence of spirochetal antibodies in deer collected in summer (54%) at the Hopland Field Station might have resulted from antigenic exposure to *B. coriaceae* instead of *B. burgdorferi* (Lane and Burgdorfer, 1986) since *O. coriaceus* ticks heavily parasitize deer during that season (Westrom, 1975). In contrast, *I. pacificus* is most abundant on deer in fall ($\bar{x} = 35.1$ ticks/deer) and least abundant on this host in summer ($\bar{x} = 0.2$ ticks/deer) (Westrom et al., 1985). Our discovery of borreliae similar to *B. coriaceae* in deer blood in summer may therefore explain not only the high seropositivity rate during that season, but the statistically significant greater prevalence of antibodies in male (53%) versus female (26%) deer since males comprised 92% (24 of 26) of the summer deer sample reported by Lane and Burgdorfer (1986). Therefore, until a more specific serologic test for spirochetal serosurveys of wildlife is available, the results of such studies should be interpreted cautiously, especially if spirochetes from both the arthropod vectors and their vertebrate hosts have not been isolated and identified.

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