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LAGOMORPHS AS SENTINELS FOR SURVEILLANCE OF BORRELIOSIS IN THE FAR WESTERN UNITED STATES

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ABSTRACT: Brush rabbits (*Sylvilagus bachmani*) and black-tailed jackrabbits (*Lepus californicus*) from California (USA) were assayed for antibodies to *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis. Significant antibody titers were detected in 90% (range, 67 to 100%) of brush rabbits from four of six localities, and in 90% of jackrabbits from a single locality, in northern California. One of the populations of brush rabbits that did not yield seropositive individuals inhabited an oceanic island devoid of any other terrestrial mammal, whereas the other population was located on an isolated flood plain bordering San Francisco Bay. Absorption tests using *B. burgdorferi* as antigen revealed that antibodies detected in both species of lagomorphs were directed against borreliae. These findings reinforce the earlier suggestion that lagomorphs may be useful as sentinel animals for surveillance of borreliosis in the far western United States.

Key words: Lyme borreliosis, spirochetes, lagomorphs, brush rabbits, jackrabbits, surveillance, survey.

INTRODUCTION

The black-tailed jackrabbit (*Lepus californicus*) is one of the most abundant and widespread lagomorphs in western North America (Hall, 1981). Recently, it was proposed that this hare may be useful as a sentinel animal for surveillance of the Lyme borreliosis spirochete (*Borrelia burgdorferi*) in California because of its wide geographic distribution, year-round breeding potential, high seropositivity rate (79%) and close association with several species of ticks known to harbor *B. burgdorferi* (Lane and Burgdorfer, 1988). Serum antibodies to *B. burgdorferi* also have been detected in *L. californicus* from Texas, and this spirochete has been isolated from a rabbit tick (*Dermacentor parumapertus*) there (Rawlings, 1986).

Cottontail rabbits (*Sylvilagus* spp.) occupy many of the same habitats and serve as hosts to many of the same tick species as jackrabbits in western North America (Kohls and Cooley, 1937; Hall, 1981; Furman and Loomis, 1984). In western California, the brush rabbit (*S. bachmani*) which also is indigenous to Baja California and Oregon, is fed upon by five species of ixodid ticks that have been found infected naturally with *B. burgdorferi* or related spirochetes in this state or elsewhere in the United States, *Dermacentor occidentalis*, *D. parumapertus*, *Haemaphysalis leporispalustris*, *Ixodes neotomae* and *I. pacificus* (Anderson and Magnarelli, 1984; Burgdorfer et al., 1985; Rawlings, 1986; Lane and Burgdorfer, 1988; Lane and Lavoie, 1988).

As part of previous investigations of the epizootiology of myxoma virus in California, serosurveys of several populations of brush rabbits were conducted in the San Francisco Bay region (e.g., Regnery and Miller, 1972). With the recent recognition that jackrabbits are significant hosts of *B. burgdorferi* or related spirochetes (Rawlings, 1986; Lane and Burgdorfer, 1988), we decided to assay brush rabbit sera obtained during the earlier myxomatosis studies for evidence of spirochetal antibodies. We also assayed blood samples from black-tailed jackrabbits for spirochetal antibodies in fall and winter since few specimens were collected during these seasons previously (Lane and Burgdorfer, 1988). Antibody absorption tests were performed on several positive brush rabbit and jackrabbit sera to confirm that the antibodies were being produced against spirochetes in the genus *Borrelia*. Finally, we attempted to isolate spirochetes from the kidneys of jackrabbits that were collected in winter and spring.

MATERIALS AND METHODS

Brush rabbits were collected at six localities in two counties in California (USA) in various months and years as follows: Alameda County—Coyote Hills (37°33'N, 122°05'W) (n = 17, July to September 1966); San Mateo County—Año Nuevo Island (37°06'N, 122°20'W) (n = 15, January (3), April (1), July (3), August (5), October (2), December (1), 1963 to 1967), Año Nuevo Point (37°07'N, 122°18'W) (n = 5, July (3), September (2), 1984), Bean Hollow (37°13'N, 122°24'W) (n = 3, July 1963, 1965), Jasper Ridge Biological Preserve (37°24'N, 122°13'W) (n = 18, March (2), August and September (15), November (1), 1965, 1970 to 1972), and Westridge (37°24'N, 122°12'W) (n = 4, July, August, 1971, 1984).

The rabbits were taken with Tomahawk live traps (Tomahawk, Wisconsin 54487, USA), transferred to a cloth bag, and bled from the lateral ear vein. Each animal was tattooed on the inner surface of one ear, weighed, sexed, examined for skin lesions and released at the site of capture. Serum was pipetted from the clotted blood and stored at −10 C.

Jackrabbits were collected by shooting at the University of California, Hopland Field Station (Mendocino County, California; 39°00'N, 123°04'W) in mid-December 1987, and in February and May 1988. Blood specimens were obtained from all of the jackrabbits taken in fall/winter, and following centrifugation serum from each was assayed for antibodies to *B. burgdorferi* as described below.

In February, kidney samples removed from two adult jackrabbits were triturated individually in 2 ml of Barbour-Stoenner-Kelly (BSK) II medium (Barbour, 1984), and 0.1 ml of the resultant suspension was inoculated into 9 ml of BSK II medium containing rifampin (50 μg/ml). In May, the left kidneys were removed from four jackrabbits (one young-of-yr, three adults) and processed similarly except that the entire organs were frozen at −75 C for ~3 mo prior to isolation attempts. After rapid thawing, a 1:10 (w/v) suspension was prepared from a tissue-sample (0.15 to 0.89 g) obtained from each kidney. One ml from each suspension was inoculated into duplicate culture tubes containing 9 ml of BSK II medium plus rifampin. Cultures were maintained at 34.5 C and examined for spirochetes by dark-field microscopy weekly for 5 wk after initial incubation periods ranging from 11 to 16 days.

Brush rabbit and jackrabbit sera were assayed for antibodies to *B. burgdorferi* (B31 strain) by indirect immunofluorescence as previously described (Lane and Burgdorfer, 1988). Experimentally prepared hyperimmune domestic rabbit serum and normal domestic rabbit serum served as positive and negative controls, respectively. A reciprocal titration end point of 64 was considered to be the minimum significant titer.

Antibody absorption tests were performed on 10 high-titered brush rabbit and jackrabbit sera using dead, whole organisms of *B. burgdorferi* (B31 strain) as antigen to absorb the spirochetal antibodies. One or two serum specimens of *S. bachmani* were tested from each of the four localities in San Mateo County that yielded seropositive brush rabbits. Sera were diluted 1:2 in PBS before antigen was added. During absorption, specimens were incubated at 37 C for 20 min. Afterward, the spirochetes were pelleted by centrifugation for 20 min (~5,000 RPM). Antibody titers for each specimen were determined pre- and postabsorption by indirect immunofluorescence. Positive and negative control sera consisted of specimens from domestic rabbits that had been hyperimmunized (titer of 1:12,800) with, or previously unexposed to, *B. burgdorferi*.

RESULTS

Antibodies to *B. burgdorferi* at titers ranging from 1:128 to 1:4,096 were detected in 67 to 100% of the brush rabbits from four of the five localities surveyed in San Mateo County (Table 1). Six of the seropositive brush rabbits were collected in 1965 from Bean Hollow (n = 2) and Jasper Ridge (n = 4). In Alameda County, none of the brush rabbits tested from Coyote Hills was seropositive, whereas in Mendocino County 9 of 10 jackrabbits had significant serum antibody titers (Table 1).

The results of the antibody absorption tests are summarized in Table 2. All 10 high-titered brush rabbit and jackrabbit sera absorbed once or twice with *B. burgdorferi* antigen were seronegative when retested.

Efforts to culture spirochetes from kidney tissues of six jackrabbits collected in February and May 1988 were unproductive.

DISCUSSION

Seropositivity rates reported here for *L. californicus* and *S. bachmani* are comparable to those reported previously for *L. californicus* (79%) from northwestern Cal-

TABLE 1. Prevalence of antibodies to *Borrelia burgdorferi* in brush rabbits (*Sylvilagus bachmani*) and jackrabbits (*Lepus californicus*) from northern California.

Species/locality	Number of sera tested	Number (%) positive	Reciprocal titration end points[a]						
			64	128	256	512	1,024	2,048	4,096
Sylvilagus bachmani									
Alameda County									
Coyote Hills	17	0							
San Mateo County									
Año Nuevo Island	15	0							
Año Nuevo Point	5	5 (100)			4	1			
Bean Hollow	3	2 (67)		1		1			
Jasper Ridge Biological Preserve	18	16 (89)		2	6	3	3	2	
Westridge	4	4 (100)				3			1
Lepus californicus									
Mendocino County									
Hopland area	10	9 (90)		2		7[b]			

[a] Indirect immunofluorescence test.
[b] Five of the seven serum specimens were not titrated to their end points.

ifornia (Lane and Burgdorfer, 1988), and are among the highest reported to date for wildlife or domestic animals in North America. In the western United States, the only other mammalian species tested to date that yielded a similar seropositivity rate has been the Barbary sheep (*Ammotragus lervia*) in Texas (Rawlings, 1986).

Human cases of Lyme borreliosis have been reported from all three counties in which lagomorphs were surveyed for spirochetal antibodies during the present study (Lane and Lavoie, 1988; R. A. Murray, pers comm.), and Mendocino is one of the most highly endemic counties in the state. The high seropositivity rates detected in brush rabbits from four disjunct mainland populations in San Mateo County, and the consistently high rates discerned in black-tailed jackrabbits from Mendocino County (Lane and Burgdorfer, 1988; present study) demonstrate that lagomorphs are significant hosts of borreliae in northern California. Further, the presence of antibodies indicates that these lagomorphs acquired their infections in diverse habitats, i.e., chaparral (Jasper Ridge), chaparral/grassland (Hopland), coastal strand (Año Nuevo Point, Bean Hollow), and suburban (Westridge). The only populations of *S. bachmani* that did not produce seropositive individuals were those inhabiting an oceanic island (Año

TABLE 2. Results of antibody absorption tests of high-titered brush rabbit and jackrabbit sera using *B. burgdorferi* B31 strain as antigen.

Species/locality	Specimen number	Reciprocal titration end points[a]	
		Pre-absorption	Post-absorption
Lepus californicus			
Hopland	RA31	≥512	<64
Hopland	33	≥512	<64
Hopland	34	≥512	<64
Hopland	35	≥512	<64
Hopland	36	≥512	<64
Sylvilagus bachmani			
Westridge	7	4,096	<64
Bean Hollow	10	512	<64
Año Nuevo Point	13	512	<64
Jasper Ridge Biological Preserve	90	1,024	<64
Jasper Ridge Biological Preserve	131	2,048	<64

[a] Indirect immunofluorescence test.

Nuevo Island) devoid of other terrestrial mammals and an isolated flood plain (Coyote Hills) bordering San Francisco Bay.

Antibody absorption test-results on sera from both species of lagomorphs confirm that the antibodies present were directed against spirochetes in the genus *Borrelia*. These findings lend further support to the earlier suggestion that lagomorphs may be useful as sentinel animals for surveillance of borreliosis in the far western United States (Lane and Burgdorfer, 1988). The high reproductive potentials of both lagomorphs (Ingles, 1965) and the fact that they serve as hosts of five species of ixodid ticks that have been found to contain *B. burgdorferi* or related borreliae (see introduction) likewise contribute to their potential value as sentinels.

The first human case of Lyme borreliosis (reported as erythema chronicum migrans) recognized in the USA occurred in Wisconsin in 1969 (Scrimenti, 1970), whereas the initial case of the disease reported from California was seen in 1975 (Naversen and Gardner, 1978). Therefore, the discovery that six brush rabbits collected in 1965 from Bean Hollow and Jasper Ridge contained significant serum antibody titers (i.e., 1:128 to 1:1,024) to *B. burgdorferi* is noteworthy. If truly such antibodies were directed against *B. burgdorferi* as suggested by the results of the antibody absorption tests, then this finding would constitute the first evidence that the Lyme borreliosis spirochete has been present in California for >20 yr. However, the results of serosurveys of wildlife unaccompanied by spirochetal isolations must be treated circumspectly until a more specific serologic test has been developed (Lane and Burgdorfer, 1986, 1988). This is particularly crucial when conducting serosurveys in regions like California where borreliae other than *B. burgdorferi* occur naturally and are known to infect wildlife, domestic animals or humans (Lane et al., 1985; Lane and Burgdorfer, 1988; Rawlings and Lane, 1988).

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