

Seasonal Prevalence of *Borrelia burgdorferi* in Natural Populations of White-Footed Mice, *Peromyscus leucopus*

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***Borrelia burgdorferi*, the etiologic agent of Lyme disease, was isolated from 111 of 237 *Peromyscus leucopus* captured during all seasons of the year. *Borreliae* were cultured from tissues of the spleen (101 mice), left kidney (76 mice), and right kidney (73 mice), from blood (12 mice), and from one fetus. Mice were infected during the winter, when immature *Ixodes dammini* were inactive. The prevalence of infection during the winter ($\leq 33\%$) was more than twofold lower than that during the summer (ca. 75%), a time when nymphal ticks are abundant. Overwintering, infected mice are reservoir hosts for subadult ticks that begin feeding in early spring. Twenty white-footed mice from which *B. burgdorferi* was isolated from tissues of spleen or kidney but not from blood were parasitized by larval *I. dammini* or *Dermacentor variabilis* which harbored borreliae. We conclude that these mice were infectious to feeding ticks, even though borreliae were not isolated from blood.**

Reservoir hosts for the Lyme disease agent *Borrelia burgdorferi* (9, 14) include mammals, birds, and ticks (1-4, 7, 9, 10, 15). While this spirochete has been frequently detected during the summer in tissues of larval ticks which have parasitized host animals and isolated in culture media inoculated with blood or tissues of mammals, no systematic study has been conducted to determine the prevalence of infected rodents throughout the year. Nor is it known whether these spirochetes survive in wild animals during the winter. Accordingly, we determined the seasonal prevalence of infected white-footed mice, *Peromyscus leucopus*, in two areas known to be endemic for Lyme disease. Parasitism of mice by *Ixodes dammini* also was recorded.

White-footed mice were captured in 1984 and 1985 in Sherman box traps in a 1-mi² (ca. 2.59 km²) rural area in East Haddam and Waterford, Conn., where *B. burgdorferi* occurs (1, 16). Ticks were removed and identified to the species level. Attempts were made to isolate borreliae from blood and from spleen and kidney tissues of 237 mice and from 5 fetuses of 5 pregnant females. A sample of blood (1 to 2 drops; 0.02 ml) or a 1:10 dilution of tissues of spleen or kidney was inoculated into duplicate tubes of Barbour-Stoener-Kelley (BSK) medium containing 0.1% agarose (SeaKem LE; FMC Corp., Marine Colloids Div., Rockland, Maine) (1, 5, 13). One of each pair of tubes contained the reducing compounds L-cystine hydrochloride (0.023%), DL-dithiothreitol (0.015%), and superoxide dismutase (0.0002%). Inoculated media were kept at 31°C for 3 to 6 weeks and examined for spirochetes by dark-field microscopy. Remaining triturated tissues were shipped overnight to the University of Minnesota, where duplicate 1:10 dilutions of the tissues were cultured at 30°C in BSK medium containing agarose. Spirochetes were identified as *B. burgdorferi* if they reacted positively in indirect fluorescent-antibody tests with monoclonal antibody H5332. This antiserum is directed against outer surface protein A of *B. burgdorferi* (6). Larval ticks that had fed on mice from which *B. burgdorferi* was isolated from spleen or kidney tissue were examined for *B. burgdorferi* by direct fluorescent-antibody staining (9).

Mice were captured during all months but were most frequently obtained from spring through fall. At East Haddam in 1984, for example, the mean numbers of mice per trap per night during January through March, April through June, July through September, and October through December were 0.2, 0.4, 0.4, and 0.4, respectively.

Although *B. burgdorferi* was isolated from *P. leucopus* during all sampling periods, we recovered this spirochete most frequently from mice captured in June through August (Table 1). During these months, the prevalence of infected mice was about 75%. The prevalence of infected mice in December, February, and March was usually equal to or less than 33%. All 111 isolates reacted with the monoclonal antibody in indirect fluorescent-antibody tests, thereby establishing their identity as *B. burgdorferi*.

Borreliae were cultured most frequently from tissues of the spleen (101 mice) and to a lesser extent from tissues of left kidneys (76 mice) and right kidneys (73 mice). Isolates from blood were made from 12 mice captured in the months of April (1 mouse), June (3 mice), July (7 mice), and August (1 mouse). One culture was obtained from a fetus of a pregnant white-footed mouse from which spirochetes also were cultured from spleen and kidney tissues.

Subadult ticks parasitized mice from April through October. Larvae first appeared in May but were most abundant in August and September. Nymphs appeared in April and were most abundant in May and June. For example, at East Haddam in 1985, mean numbers \pm one standard deviation of larvae per mouse by month from May through September were 2.4 \pm 4.1, 1.1 \pm 1.3, 0.9 \pm 1.5, 9.9 \pm 13.5, and 21.0 \pm 25.4, respectively. For nymphs collected from April through August, mean numbers per mouse by month were 0.1 \pm 0.4, 0.9 \pm 1.9, 1.4 \pm 1.3, 0.2 \pm 0.4, and 0.4 \pm 0.5, respectively.

Twenty white-footed mice from which *B. burgdorferi* was isolated from tissues of spleen or kidney but not from blood were parasitized by one or more larval *I. dammini* or *Dermacentor variabilis* that harbored borreliae. Five of these mice were parasitized only by *D. variabilis* larvae and nine mice were parasitized by 2 to 13 larval *I. dammini* that carried spirochetes. One mouse was parasitized by infected larvae of both tick species. On the basis of these results, we conclude that mice which yielded cultures of *B. burgdorferi*

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TABLE 1. Prevalence of white-footed mice infected with *B. burgdorferi* in East Haddam and Waterford, Conn., during 1984 and 1985^a

Mo	East Haddam					Waterford				
	No. of borrelia-positive mice/no. examined	No. of isolations from:				No. of borrelia-positive mice/no. examined	No. of isolations from:			
		Blood	Spleen	Left kidney	Right kidney		Blood	Spleen	Left kidney	Right kidney
June 1984	ND	ND	ND	ND	ND	2/5	0	1	0	1
July	8/10	5	5	5	5	ND	ND	ND	ND	ND
August	2/3	0	2	1	1	ND	ND	ND	ND	ND
September	0/3	0	0	0	0	3/7	0	3	1	0
October	4/11	0	3	1	3	2/6	0	2	2	2
November	4/6	0	4	3	3	ND	ND	ND	ND	ND
December	3/13	0	2	2	1	1/12	0	1	0	0
February 1985	2/6	0	2	1	2	4/12	0	3	2	2
March	2/10	0	0	1	1	2/12	0	2	1	1
April	4/11	1	4	2	3	4/10	0	3	3	1
May	8/14	0	8	5	7	9/23	0	9	7	6
June	11/14	3	11	10	10	9/10	0	9	7	5
July	5/9	0	5	5	5	8/10	2	8	7	5
August	6/8	0	6	3	3	8/10	1	8	7	6
September	0/2	0	0	0	0	ND	ND	ND	ND	ND
Total	59/120	9	52	39	44	52/117	3	49	37	29

^a Determined by isolation of borreliae in BSK medium and their reaction with monoclonal antibody H5332 in indirect fluorescent-antibody tests. ND, Not done.

from tissues of spleen or kidney but not from blood were infectious to feeding ticks and may therefore be spirochetemic, even though spirochetes were not isolated from blood.

B. burgdorferi previously has been shown to survive during the winter in *I. dammini* (4). These spirochetes also are present throughout the winter in white-footed mice. Although it is unknown how long these animals retain spirochetes, laboratory studies have shown a persistence of *B. burgdorferi* in white-footed mice for up to 2 months, and in hamsters, infection lasted at least 9 months (1, 12, 13). Therefore, mice harboring spirochetes in the winter may have acquired *B. burgdorferi* from nymphal tick bites from April through August of the previous calendar year. However, the acquisition of new infections during the winter cannot be ruled out, because transmission may also occur by direct contact among mice (8, 11).

The increase in infection rates among mice in June corresponds to parasitism by nymphal *I. dammini*, which are most abundant from May through June (17, 18). Clearly, many mice harbor spirochetes and are therefore reservoirs during summer through September. Larval ticks are most abundant from July to September and become infected while feeding on these animals (4, 7, 15). The decline in numbers of infected mice in the fall and winter is probably due to decreased exposure to nymphal ticks, the appearance of new broods of mice which have not been parasitized by ticks, and the natural mortality of older mice (19). Nevertheless, some mice are infected with these bacteria during the winter and remain as reservoirs for subadult ticks, which begin feeding in early spring.

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