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Serologic Analyses of *Peromyscus leucopus*, a Rodent Reservoir for *Borrelia burgdorferi*, in Northeastern United States

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An enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent-antibody test were used to detect antibodies to *Borrelia burgdorferi*, the causative agent of Lyme disease, in *Peromyscus leucopus* (white-footed mouse). Of the 661 mice captured in Connecticut, Rhode Island, and New York during 1980 and 1983 to 1987, 166 (25.1%) had antibodies to *B. burgdorferi* by ELISA. Comparative analyses of 210 serum specimens, collected in areas where Lyme disease is endemic, revealed a threefold difference in sensitivity between the ELISA (38.1% positive) and the indirect fluorescent-antibody method (12.4%). Although prevalence of seropositive *P. leucopus* was highest during June, elevated amounts of antibody (1:1,280 to 1:2,560) were detected in mice that harbored spirochetes during all seasons. Being reservoirs for *B. burgdorferi*, these rodents are suitable for monitoring spirochete infections at foci and should be included in field evaluations of control programs aimed at suppressing Lyme disease.

The etiologic agent of Lyme disease, *Borrelia burgdorferi*, is transmitted by ticks of the *Ixodes ricinus* complex to mammals and birds. In northeastern United States, Wisconsin, and Minnesota, *Ixodes dammini* is the primary vector (2, 3, 5, 7, 12, 15, 18). Although in its immature stages this hard-bodied tick is known to feed on a variety of vertebrate hosts (6, 13, 20, 25), including rodents, humans, domestic animals, and passerine birds, *Peromyscus leucopus* (the white-footed mouse) is considered a chief reservoir for *B. burgdorferi* in or near woodlands (1, 2, 5, 10, 11, 14, 17). The detection of antibody to this bacterium (15, 20, 21), frequent isolation of *Borrelia* spirochetes in areas where Lyme disease is endemic (1, 2, 4, 5), and the ability of *P. leucopus* to infect immature *I. dammini* (14, 17) reinforce the epidemiological significance of this mammal.

The association of *I. dammini* larvae and nymphs with *P. leucopus* is well documented (6, 13, 15, 20, 25). It is, therefore, appropriate to select this mammal for surveillance programs. Indirect fluorescent-antibody (IFA) methods have been used to detect antibody to *B. burgdorferi* (15, 20), but among the mammals studied, the prevalence of seropositive white-footed mice has been relatively low. It is unclear whether these rodents have weak immunologic responses to *B. burgdorferi* or whether the results are due to low test sensitivity. Accordingly, this investigation was conducted to further evaluate an enzyme-linked immunosorbent assay (ELISA) developed earlier (24), to compare results with those obtained by IFA, and to determine whether ELISA is more suitable for monitoring immunologic responses to *B. burgdorferi* in *P. leucopus* populations.

MATERIALS AND METHODS

Sampling. White-footed mice were captured in Sherman box traps from woodlands in Connecticut, Rhode Island, and New York during 1980 and 1983 to 1987. Although rodents were collected during all seasons, the majority were obtained from May through August. Sites in Connecticut (East Haddam, Norwich, and Salem) are located in the south-central or southeastern part of the state, a region where Lyme disease is prevalent in human populations (21, 28). Mice were marked, released, and recaptured at East Haddam as previously described (20). In Rhode Island, *P. leucopus* was collected from inland areas, Block Island, Prudence Island, and Jamestown Island, whereas in New York, animals were captured in Westchester and Dutchess Counties. All study sites are within the geographical range of *I. dammini*. For controls, 60 additional mice were obtained from Newtown and Woodbridge, Conn., towns where Lyme disease and *I. dammini* are rare.

Serologic tests. Details on the use of IFA and ELISA methods and on quantitating total immunoglobulins to *B. burgdorferi* have been reported previously (20, 24). To improve the sensitivity of the ELISA, the concentration of washed, whole-cell antigen used to coat the solid phase was increased from 7 μg of protein per ml to 15 μg of protein per ml. In addition, the dilution of unconjugated rabbit anti-*P. leucopus* immunoglobulins was increased from 1:200 to 1:400, and the concentration of horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G antibodies in phosphate buffer solutions was changed from 1:800 to 1:2,500. With these modifications, test sera were screened at dilutions ≥1:160; net absorbance values (i.e., net optical densities) greater than 0.39, 0.34, and 0.29 were graded as positive for the respective serum dilutions of 1:160, 1:320, and ≥1:640. These critical regions were determined by statistical analyses (3 standard deviations + mean) of net absorbance values for the respective serum dilutions of 60 normal specimens. Positive controls included serum samples from *P. leucopus* that had been found harboring *B. burgdorferi*. Procedures for blocking nonspecific binding sites and for the preparation of diluents, washes, and substrate remained unchanged. When new lots of enzyme-labeled antibodies or other reagents were purchased, tests were reevaluated with positive and negative serum controls and

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TABLE 1. Comparison of IFA and ELISA results for *P. leucopus* sera

Site	Yr	Total no. of sera tested	IFA		ELISA	
			No. (%) positive	Antibody titer[a] (range)	No. (%) positive	Antibody titer[a] (range)
Norwich, Conn.	1986	50	0		6 (12)	320–2,560
Salem, Conn.	1986	135	23 (17)	32–128	69 (51.1)	320–2,560
Rhode Island	1983–1985	25	3 (12)	32–512	5 (20)	320–1,280

[a] Reciprocal antibody titers ≥32 (IFA positive) and ≥320 (ELISA positive).

procedures were standardized accordingly. Uniformity of assay sensitivity was monitored daily by including the same positive and negative control sera on each plate. Controls were also included for antigens, conjugates, buffer solutions, and diluents.

To assess the specificity of the IFA method, 8 serum samples with antibodies to *B. burgdorferi* and 12 specimens that were nonreactive to this spirochete were screened against *Borrelia hermsii* (a relapsing fever spirochete), *Treponema pallidum* (the causative agent of syphilis in humans), and the following serovars of *Leptospira interrogans*: canicola, icterohaemorrhagiae, and pomona. The sources and preparation of these antigens have been reported previously (19).

Isolations. The blood, spleen tissues, or kidney tissues of white-footed mice were aseptically removed and processed for spirochete isolations as described previously (1, 5, 16). Briefly, 20 μl of blood or 1:10 dilutions of spleen and kidney tissues, triturated in 7 ml of Barbour-Stoenner-Kelly medium, were placed into duplicate tubes of this medium containing 0.1% agarose. Cultures were kept at 31°C for 3 to 6 weeks and examined for spirochetes by dark-field microscopy. Isolates were subcultured and identified as *B. burgdorferi* by fixing washed, whole cells to glass microscope slides and by applying murine monoclonal antibody (H5332) and appropriate conjugates in IFA tests (8, 9). This monoclonal antibody was directed against outer surface protein A, a polypeptide with an approximate molecular mass of 31 kilodaltons. This protein is common to North American isolates of *B. burgdorferi*. Results verifying the presence of *B. burgdorferi* in rodents and arthropods from sites in Connecticut, Rhode Island, and New York have been published before (1, 2, 5, 7, 12, 19).

Statistical analyses. To determine significant differences in percentages of positive sera, values were analyzed by a Student *t* test after arcsin transformation (27).

RESULTS

Comparative analyses of 210 *P. leucopus* sera revealed threefold greater sensitivity by modified ELISA (Table 1). In areas where Lyme disease is prevalent in human populations, the number of positive serum samples by ELISA (n = 80) greatly exceeded that determined by the IFA method (n = 26). Antibody titers were also correspondingly higher in the ELISA than in the IFA test. In analyses of an additional 24 serum samples, representing *P. leucopus* specimens from areas where *I. dammini* and Lyme disease are rare, results were negative in both assays. To assess reproducibility of the ELISA, 24 positive sera were retested. There were no differences in antibody titers for 10 samples, whereas twofold (n = 12) or fourfold (n = 2) variations were recorded for the remaining sera in the second trial.

White-footed mice collected in Connecticut, Rhode Island, and New York (Westchester County) contained antibodies to *B. burgdorferi*. However, prevalence of seropositive animals by ELISA was highly variable (Table 2). For example, in 1984 and 1985 the number of *P. leucopus* specimens with antibodies in East Haddam, Conn., differed by nearly threefold. During 1986, more than half of the 135 *P. leucopus* specimens captured in Salem, Conn., had been exposed to Lyme disease spirochetes. Antibody titers ranged from 1:320 to 1:2,560, and among these, 1:640 was recorded most frequently (40.7% of 172 positive sera) followed by 1:320 (23.3%), 1:1,280 (21.5%), and 1:2,560 (14.5%).

Although antibodies to *B. burgdorferi* were present in *P. leucopus* during all months of the year in East Haddam, Conn. (Table 3), there were significantly more positive sera collected during June through August (22%) than during March through May (12%) (t = 2.1, $P < 0.03$). Antibody titers varied during all seasons, but titration endpoints peaked (1:2,560) during May, July, and August.

TABLE 2. Prevalence of *P. leucopus* specimens with antibodies to *B. burgdorferi*

Site and yr	No. of serum samples tested	No. (%) positive[a]	No. of specimens with reciprocal titer of:			
			320	640	1,280	2,560
East Haddam, Conn.						
1980	147	25 (17)	8	12	3	2
1983	63	11 (17.5)	2	7	1	1
1984	81	9 (11.1)	2	6	1	0
1985	61	18 (29.5)	3	6	7	2
Salem, Conn.						
1986	135	69 (51.1)	19	25	13	12
1987	25	6 (24)	2	2	0	2
Norwich, Conn., 1986	50	6 (12)	2	2	1	1
Rhode Island, 1983–1985, 1987	57	10 (17.5)	1	3	5	1
Westchester County, N.Y., 1986	47	18 (38)	1	7	6	4
Dutchess County, N.Y., 1986	20	0				
Total	686	172 (25.1)	40	70	37	25

[a] Analyzed by ELISA.

TABLE 3. Prevalence of white-footed mice with antibodies to *B. burgdorferi* in East Haddam during 1980 and 1983 to 1985

Mo	Total sera tested	No. (%) ELISA positive[a]	Reciprocal antibody titer	
			Range	Geometric $\bar{x}$
January	12	1 (8.3)	640	
February	10	2 (20)	640–1,280	905
March	19	3 (15.8)	320–640	508
April	41	3 (7.3)	320–1,280	640
May	40	6 (15)	320–2,560	718
June	58	18 (31)	320–1,280	616
July	54	9 (16.7)	320–2,560	941
August	47	8 (12.8)	320–2,560	761
September	39	7 (18)	320–1,280	640
October	14	2 (14.3)	320–640	453
November	16	3 (18.8)	640–1,280	806
December	2	1 (50)	640	
Total	352	63 (17.9)	320–2,560	630

[a] Prevalences based on sample sizes <19 not included in statistical comparisons.

Multiple serum samples were obtained from 82 *P. leucopus* specimens in East Haddam during 1980, 1983, and 1984. Seroconversions (i.e., a negative-to-positive change in test results) were recorded for 6 mice from May through September (Table 4). Rising antibody titers were detected in 4 animals by ELISA, but IFA test results for these sera remained negative. The second or subsequent serum specimens from the remaining 76 mice were negative in either or both assays.

Infected white-footed mice had antibodies to spirochetes during all seasons. Of the 206 animals tested for *B. burgdorferi*, 72 (35%) harbored this bacterium in their blood, kidneys, or spleen (Table 5). The majority of these (57.8% of 72) had detectable amounts of antibody to *B. burgdorferi* with titers ranging from 1:320 to 1:2,560 in ELISA. Although spirochetes were not isolated from the remaining 134 *P. leucopus* specimens, antibodies were detected in similar amounts in 10 mice.

Serum antibodies from 8 mice collected in Salem, an area where Lyme disease is highly endemic, cross-reacted in IFA tests against *B. hermsii* and *T. pallidum*. Similar tests with serovars of *L. interrogans* were negative. Antibody titers to *B. hermsii* differed twofold or less from homologous reactions to *B. burgdorferi* in all serum samples, whereas titration endpoints to *T. pallidum* were at least fourfold lower than those to the Lyme disease agent. All 12 serum specimens without antibodies to *B. burgdorferi* were likewise negative when tested against all other spirochetal antigens.

TABLE 5. Prevalence of *Borrelia*-infected *P. leucopus* specimens with antibodies to spirochetes

Site	Yr	Total no. infected[a]	No. (%) of infected mice[b]	
			With antibodies	Without antibodies
East Haddam, Conn.	1984[c]	6	4 (67)	2 (33)
East Haddam, Conn.	1985[c]	33	14 (42.4)	19 (57.6)
Rhode Island	1984–1986[d]	6	4 (67)	2 (33)
New York	1986[e]	27	16 (59.3)	11 (40.7)
Total		72	38 (57.8)	34 (47.2)

[a] Some isolates reported earlier (1, 2, 5).
[b] Tested by ELISA.
[c] Increase in isolations due to improved growth medium and the testing of spleen and kidney tissues.
[d] Mice from Prudence Island.
[e] Mice from Westchester County.

DISCUSSION

Antibodies to *B. burgdorferi* can be detected by either an IFA test or a modified ELISA, but the latter had greater sensitivity. The relatively low number of seropositive *P. leucopus* specimens reported earlier (20) was a conservative estimate of *B. burgdorferi* presence in endemic areas. Therefore, for surveillance purposes, ELISA is preferred because of greater sensitivity and ease of automation and standardization and because test results are more objective.

White-footed mice can harbor *B. burgdorferi* during all seasons. Anderson et al. (1) reported infection rates of ≥66% during the summer and ≤33% during the winter. In addition, the number of mice with antibodies to the Lyme disease agent varied seasonally and geographically. The highest titers of antibody and number of seroconversions occurred during the summer, a time when nymphs of *I. dammini* were active. Therefore, the warmer months appear to be most suitable for monitoring serologic responses to *B. burgdorferi* in these rodents.

Antibodies to *B. burgdorferi* were present in mice that had spirochete infections. Compared with those for other mammals, such as humans or dogs, antibody titers for these rodents were relatively low, regardless of the assay method used. Since some infected mice had no detectable antibody, immunologic response in *P. leucopus* may be delayed during initial and possibly late stages of infection. Even when immunoglobulins are produced, we suspect that they may be relatively ineffective in quickly neutralizing the spirochetes. Reservoir competence of *P. leucopus* has been confirmed (14, 17), and although spirochetemia periods are unknown, these and possibly other vertebrate hosts (2, 3, 6) are

TABLE 4. Antibody titers for *P. leucopus* specimens recaptured in East Haddam, Conn., during 1980 and 1983

Mouse no.[a]	Date of capture	Reciprocal antibody titer at capture		Date of recapture	Reciprocal antibody titer at recapture	
		IFA	ELISA		IFA	ELISA
1	14 May 1980	Neg[b]	Neg	23 July 1980	Neg	640
2	7 August 1980	Neg	Neg	20 August 1980	Neg	640
3[c]	25 April 1983	Neg	—[d]	10 May 1983	1:32	—
4	19 May 1983	Neg	—	9 June 1983	Neg	640
5	9 June 1983	Neg	Neg	24 June 1983	Neg	1,280
6	9 September 1983	Neg	Neg	29 September 1983	—	320

[a] Isolation of spirochetes not attempted.
[b] Neg, Negative.
[c] Additional serum specimens collected on 19 April (IFA, negative) and 19 May (ELISA, 1:640).
[d] —, Not tested because of insufficient amounts of serum.

important sources of infection. Transovarial transmission of *B. burgdorferi* occurs at a low rate in *I. dammini* (22, 26). Therefore, uninfected larval or nymphal ticks acquire *B. burgdorferi* by feeding on spirochetemic hosts and transmit the spirochetes when they refeed in later life stages.

White-footed mice are abundant in woodlands, have relatively small home ranges, are easily caught, and often have numerous larvae and nymphs of *Dermacentor variabilis* and *I. dammini* attached (2, 6, 13, 15, 20, 25). Antibodies produced against *B. burgdorferi* are now readily detected by ELISA, and methods are available for isolating this pathogen. Therefore, *P. leucopus* is ideal for studies designed to provide more precise information on the distribution and prevalence of *B. burgdorferi* infections in nature. Serologic analyses for the Lyme disease agent should be conducted along with isolation procedures. Furthermore, with growing interest in finding ways to decrease *I. dammini* populations and to reduce the risk of human infection, white-footed mice should be included in field evaluations of control efforts.

Tests on the specificity of our IFA method revealed cross-reactivity among the *Borrelia* and *Treponema* species. This has been demonstrated before (19, 23) for antibodies in sera from humans and white-tailed deer (*Odocoileus virginianus*). However, since tick-borne relapsing fever is not known to occur in eastern United States and since *T. pallidum* does not infect *P. leucopus*, cross-reactivity with *B. burgdorferi* is probably insignificant. However, other unclassified spirochetes have been isolated from *P. leucopus* specimens and short-tailed shrews (*Blarina brevicauda*) in Connecticut and Minnesota (4). These organisms appear to be serologically distinct from species of *Borrelia*, *Treponema*, *Leptospira*, and *Spirochaeta*. Nonetheless, there might be other unknown spirochetes in nature that could stimulate immune responses in *P. leucopus* and lessen the specificity of these assays. Therefore, serologic evidence of *B. burgdorferi* infections should be supported by isolation and characterization of spirochetes in study areas.

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