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Antibodies to *Borrelia burgdorferi* in Rodents in the Eastern and Southern United States

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Serologic studies were conducted to determine whether white-footed mice (*Peromyscus leucopus*) and cotton mice (*Peromyscus gossypinus*) contained serum antibodies to *Borrelia burgdorferi*, the causative agent of Lyme borreliosis. Enzyme-linked immunosorbent assays detected antibodies to this spirochete in 35.7 and 27.3% of 56 *P. leucopus* and 535 *P. gossypinus* serum samples, respectively, collected in Connecticut, North Carolina, South Carolina, Georgia, Florida, Alabama, and Mississippi. Antibody titers ranged from 1:160 to ≥1:40,960. On the basis of adsorption tests, the antibodies detected appeared to be specific to *Borrelia* spirochetes. Seropositive rodents in the eastern and southern United States, areas where human cases of Lyme borreliosis have been reported, indicate a widespread geographic distribution of *B. burgdorferi* or a closely related spirochete.

Human cases of Lyme borreliosis have been reported in widely separated regions of the United States. Although numbers of documented cases are comparatively higher in northeastern and upper midwestern states, human infections of this tick-associated illness also occur in southeastern states (29).

Known or suspected tick vectors in the eastern United States include *Ixodes dammini*, *Ixodes scapularis*, and *Amblyomma americanum* (1, 8, 9, 17, 23, 24). The presence of *I. dammini* in northeastern and midwestern states, areas of high endemicity for Lyme borreliosis, is well established (1, 8, 10, 18, 21, 27). Isolations of *Borrelia burgdorferi* from this tick, rodents, and human beings in these regions (3–6, 8, 13, 28) provide confirmatory evidence. In North Carolina, *B. burgdorferi* has been detected in midgut tissues of *I. scapularis* and *A. americanum* by indirect fluorescent-antibody staining methods (17), but information on the presence of this spirochete in rodents there and in other southern states is limited.

Rodents, particularly *Peromyscus leucopus* (the white-footed mouse), are important hosts for immature ticks (1, 10, 15, 18, 21) and can harbor *B. burgdorferi* for several weeks (3, 4, 6, 11, 12, 22). These and other mammals produce antibodies (16, 17–19, 25) to this bacterium. The main objective of this study was to use an enzyme-linked immunosorbent assay (ELISA) to detect antibodies in *P. leucopus* and to determine by adsorption procedures and further serologic testing whether antibodies in these rodents and in *Peromyscus gossypinus* are specific to *Borrelia* species.

MATERIALS AND METHODS

Study sites and sampling. Blood samples were obtained during the period from 1986 to 1990 from *P. leucopus* and *P. gossypinus* in Connecticut, North Carolina, South Carolina, Georgia, Florida, Alabama, and Mississippi. Most collection sites were located on the mainland, but areas off the coasts of Georgia (Sapelo and Cumberland Islands) and Florida (Amelia Island) were included. Since the prevalence of *I. dammini* infected with *B. burgdorferi* can be high on islands (8), it was especially appropriate to investigate these sites. Rodents were captured in Sherman box traps (18) and anesthetized prior to obtaining blood samples. All sera were stored at −60°C until analyses.

Serologic tests. An ELISA was used to detect immunoglobulins to *B. burgdorferi* and to determine antibody concentrations. Details on the materials and methods used have been reported previously (16, 19). To save time in laboratory testing, a newly developed affinity-purified horseradish peroxidase-labeled goat anti-*P. leucopus* immunoglobulin (heavy and light chain specific) replaced the unconjugated rabbit anti-*P. leucopus* immunoglobulins and the peroxidase-conjugated goat anti-rabbit reagent used previously. The commercially prepared peroxidase-labeled anti-*P. leucopus* antibodies (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) were diluted 1:1,000 to 1:5,000 in phosphate-buffered saline (PBS) solution and tested against a series of positive and negative serum samples to determine the optimal working concentration (1:2,000). Net optical density values for the modified ELISA were 0.18, 0.15, and 0.11 for the respective serum dilutions of 1:160, 1:320, and ≥1:640. These critical regions for positive test results were computed by statistically analyzing (3 standard deviations + mean) net absorbance readings for 38 normal specimens. The original ELISA procedures (19), with polyvalent reagents, were used to test *P. gossypinus* sera. Positive control sera were obtained from naturally infected *P. leucopus* mice (i.e., culture positive for *B. burgdorferi*) collected in Lyme, Conn. Negative controls were from white-footed mice captured in northwestern Connecticut, where *I. dammini* and Lyme borreliosis are rare. Uniformity of assay results was assured by including the same positive and negative serum controls and by performing tests for reproducibility. Additional controls were included for antigen, conjugates, and diluents.

Specificity studies. Rodent serum samples from Connecticut (n = 16), North Carolina (n = 21), Georgia (n = 21), and

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TABLE 1. Prevalence of *P. gossypinus* and *P. leucopus* with antibodies to *B. burgdorferi* in the eastern United States, 1986 to 1990

State	*P. gossypinus*		*P. leucopus*	
	No. of serum samples tested[a]	No. (%) positive	No. of serum samples tested[a]	No. (%) positive
Connecticut[b]	0		16	11 (68.8)
North Carolina	69	15 (21.7)	30	9 (30.0)
South Carolina	29	11 (37.9)	0	
Georgia	144	53 (36.8)	0	
Florida	103	36 (35.0)	0	
Alabama	80	12 (15.0)	0	
Mississippi	110	19 (17.3)	10	0
Total	535	146 (27.3)	56	20 (35.7)

[a] Sera tested by polyvalent ELISA.
[b] Lyme, Old Lyme, and East Lyme, Conn.

Florida (n = 5) were screened by indirect fluorescent-antibody staining methods for antibodies to *Treponema pallidum* and *Leptospira interrogans* serovar canicola. Serum samples with (n = 40) or without (n = 23) antibodies to *B. burgdorferi* were included in these analyses. The sources and preparations of antigens have been reported previously (17). Polyvalent fluorescein-conjugated rabbit anti-*P. leucopus* (18) immunoglobulins were diluted 1:20 in PBS solution. Rabbit antisera with homologous antibodies to these spirochetes (17) were used to verify antigen reactivity. In addition, adsorption procedures were used to further assess specificity. Sorbents included a 1:2 dilution of *Treponema phagedenis* biotype Reiter (SciMedx Corp., Denville, N.J.) or washed cells of *B. burgdorferi* or *L. interrogans* serovar canicola. Details on mixing sorbent with test sera and subsequent incubation and analyses have been reported previously (20).

RESULTS

Serum samples collected in seven states contained antibodies to *B. burgdorferi* (Table 1). Seropositivity for *P. leucopus* ranged from 30.0 to 68.8% in North Carolina and Connecticut, respectively. *P. gossypinus* also contained antibodies to this spirochete; seropositivity was highest (37.9%) in South Carolina. The geometric mean ($\bar{x}$ = 1,076) for *P. leucopus* captured in Connecticut exceeded all mean values for this species and *P. gossypinus*.

P. gossypinus was exposed to *B. burgdorferi* or a closely related spirochete in six states, though not in every county investigated. Moreover, seropositivity and geometric mean antibody titers were highly variable in each state (Table 2). Although antibody titers normally ranged between 1:160 and 1:2,560, maximal titration endpoints of 1:10,240 and ≥1:40,960 were recorded for samples collected in North Carolina, Georgia, and Alabama. Geometric mean antibody titers were comparatively higher for sera collected in Ossabaw Island, Georgia ($\bar{x}$ = 147.7), Sapelo Island, Georgia ($\bar{x}$ = 427.9), and Amelia Island, Florida ($\bar{x}$ = 597.1).

Additional analyses were conducted with *P. leucopus* sera to determine whether there was comparable sensitivity in an ELISA with or without the newly developed peroxidase-labeled goat anti-*P. leucopus* immunoglobulins. Of the 24 positive serum samples tested by the original ELISA, 20 (83%) specimens remained positive when retested with the new antibody reagent. Titration endpoints ranged from 1:320 to 1:5,120 in the original ELISA and from 1:320 to 1:40,960 by the modified ELISA. The remaining four samples were negative when the new reagent was used. In similar tests of an additional 24 serum samples, which were also collected from animals in tick-infested areas but found to be negative in the original ELISA, results for 17 serum samples were unchanged when reanalyzed with the new reagent. Seven samples were positive with titers of 1:640 and 1:2,560 by the newly developed ELISA.

Serum samples from 37 white-footed mice and 26 cotton mice, with or without antibodies to *B. burgdorferi*, were retested by indirect fluorescent-antibody staining methods for reactivity to *T. pallidum* and *L. interrogans* serovar canicola. Of the 40 mouse serum samples with antibodies (1:128 to 1:8,192) to *B. burgdorferi*, two reacted to *T. pallidum* and the *Leptospira* serovar (1:64). Antibody titers to *B. burgdorferi* were at least eightfold greater than those to the other spirochetes. Twenty-three serum samples, lacking antibodies to *B. burgdorferi*, were nonreactive to *Treponema* and *Leptospira* spirochetes when a serum dilution of 1:64 was tested.

To further evaluate specificity, washed whole cells of *B. burgdorferi* and *L. interrogans* serovar canicola and commercial preparations of *T. phagedenis* biotype Reiter sorbent were used to remove homologous or heterologous antibodies from the sera. The addition of *B. burgdorferi* (30 μg of protein per ml) to two cotton mouse serum samples, which were reactive to all spirochetes, completely removed antibodies to *Treponema* and *Leptospira* spirochetes (i.e., negative at a serum dilution of 1:64) and caused a four- to eightfold drop in antibody concentration to *B. burgdorferi*. Nonetheless, seropositivity to *B. burgdorferi* was maintained (titers = 1:64 to 1:512) for each sample following adsorption. In duplicate tests, treatment of these samples with a 1:2 dilution of *T. phagedenis* sorbent reduced titers to *B. burgdorferi* and to *T. pallidum* by twofold and completely removed antibodies to the *Leptospira* serovar. Following the application of washed cells of *L. interrogans* serovar canicola (30 μg of protein per ml) in a third test, titration endpoints to *Borrelia*, *Treponema*, and *Leptospira* spirochetes were unchanged.

DISCUSSION

On the basis of serologic studies, the mice in the present study were exposed to *B. burgdorferi* or a closely related spirochete at widely separated sites in the eastern and southern United States. Tick-borne relapsing fever spirochetes, such as *Borrelia hermsii*, are not known to occur in the eastern United States (7). Tests for *Leptospira* and *Treponema* antibodies and adsorption trials indicate that the antibodies detected in mice were probably against *B. burgdorferi*. However, the number of well-documented cases of Lyme borreliosis in humans is relatively low in the southern United States, the tick vector has not been clearly identified there, and there are no reports of spirochetes cultured from and properly characterized for ticks or mammals, verifying the presence of *B. burgdorferi*. Therefore, further studies are required to confirm Lyme borreliosis infections, particularly in humans, by isolation and immunochemical characterization procedures. Results of the present study can be used to help locate communities for such isolation work. Also, there appears to be a complex of closely related strains of *B. burgdorferi* in the northeastern United States. Some strains cause arthritis in Lewis rats and other laboratory rodents, while others do not (2). Once isolations of *B. burgdorferi*

TABLE 2. Prevalence of *P. gossypinus* with antibodies to *B. burgdorferi* in the eastern and southern United States, 1988 to 1990

State and county	Sampling date(s)	No. of serum samples tested by ELISA	No. (%) positive	Reciprocal antibody titer	
				Geometric $\bar{x}^a$	Range
North Carolina					
Bertie	28 Apr. 1990	11	0	40.0	
Onslow	30 June 1990	8	1 (12.5)	67.3	2,560
Gates	27 Apr. & 16 June 1990	45	12 (26.7)	81.3	160–10,240
New Hanover	18 July 1988	5	2 (40.0)	100.5	640–1,280
South Carolina, Marion & Dillon	7 May 1990	29	11 (37.9)	104.1	160–640
Georgia					
Glynn	1 July 1988	2	0	40.0	
Chatham	3 July 1988	3	0	40.0	
Upson & Pike	22 Apr. 1990	8	0	40.0	
McDuffie	13 Apr. 1990	37	11 (29.7)	51.4	160–640
Clinch	22 June 1989	4	1 (25.0)	56.6	160
Camden	6 Aug. 88 & 14 May 1990	19	3 (15.8)	64.3	320–2,560
Camden (Cumberland Island)	13 May 1990	14	3 (21.4)	99.1	160–640
Bryan (Ossabaw Island)	21 Aug. 1988 & 16 May 1989	26	15 (57.7)	147.7	160–2,560
McIntosh (Sapelo Island)	17 July & 1 Sept. 1989	31	20 (64.5)	427.9	320–>40,960
Florida					
Putnam, Clay, & St. Johns	30 July 1988	18	2 (11.1)	54.4	640
Santa Rosa & Okaloosa	31 Aug. 1988	24	4 (16.7)	61.7	320–640
Nassau	27 July & 7 Aug. 1988	13	3 (23.1)	64.6	160–640
Alachua	28 July 1988	5	2 (40)	105.6	320–640
Jackson & Leon	3 July 1989	33	16 (48.5)	135.3	160–2,560
Nassau (Amelia Island)	23–24 June 1989	10	9 (90)	597.1	160–5,120
Alabama					
Tuscaloosa	16 Aug. 1988	14	0	40.0	
Calhoun	5 Jan. 1989	10	1 (10)	49.2	320
Barbour	19 May 1990	37	7 (18.9)	62.7	160–2,560
Washington & Clarke	21 May 1990	19	4 (21.1)	86.1	320–10,240
Mississippi					
Sharkey	28 Aug. 1988 & 24 May 1990	35	2 (5.7)	45.0	160–640
Stone & Perry	30 Aug. 1988	15	3 (20.0)	66.5	320–1,280
Franklin	23 May 1990	47	11 (23.4)	67.0	160–2,560
Tishomingo & Prentiss	25 May 1990	13	3 (23.1)	80.0	160–5,120

[a] A value of 40, average reciprocal titer for negative sera, was used for each negative sample in analyses.

have been made in the southern states, comparative studies on the pathogenicity of these strains should be conducted.

The degree of exposure of mice to *B. burgdorferi* varies geographically. Percentages of mice with antibodies to *B. burgdorferi* in Alabama and Mississippi were relatively lower than those computed for these hosts in other states. Moreover, geometric mean antibody titers were particularly high for mice captured in Connecticut and islands off the coast of Georgia (Ossabaw and Sapelo) and Florida (Amelia). These results may be due, in part, to higher populations of ticks in settings where these rodents and white-tailed deer (*Odocoileus virginianus*) abound. Risk of exposure of humans to infected ticks may likewise be greater in these sites. Although *I. scapularis* and *A. americanum* in Alabama harbor *B. burgdorferi* (14), the prevalence of spirochetemic mice and infected ticks there and in other southern states needs further assessment.

Utilization of an ELISA with newly developed peroxidase-labeled goat anti-*P. leucopus* immunoglobulins is an improvement over earlier procedures. Less time is required to perform the assay, and sensitivity is maintained. The availability of this new reagent also makes it possible to perform Western blot (immunoblot) analyses. Immunoblotting techniques have been helpful in the serologic diagnosis of Lyme borreliosis in humans (27). Specific banding patterns, such as reactivity to outer surface protein A (31-kDa polypeptide), can help confirm *B. burgdorferi* infections (27). In more recent work (26), a 39-kDa polypeptide appears to be another relatively specific immunodominant antigen of this spirochete. These and other antigens of higher molecular masses might be immunologically recognized by mice. Therefore, in the absence of isolation results, immunoblots of sera from white-footed mice and cotton mice may provide additional supportive evidence of *B. burgdorferi* presence in mid-Atlantic and southern states.

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