IgG Antibodies to *Borrelia burgdorferi* in Rodents in Tennessee

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ABSTRACT

Goat-anti white-footed mouse (*Peromyscus leucopus*) IgG was used in an enzyme-linked immunosorbent assay (ELISA) and a Western blot assay to test for borrelial antibodies in sera from cricetid rodents captured in Tennessee. The conjugate was cross-reactive between cricetids (New World rodents) but was weakly or not cross-reactive with murids (Old World rodents) and sciurids (squirrels). Using the ELISA, 9% of cotton rats (*Sigmodon hispidus*), 19% of woodland voles (*Microtus pinetorum*), 31% of white-footed mice (*Peromyscus leucopus*), 57% of golden mice (*Ochrotomys nuttalli*), and 100% (one captured) of cotton mice (*P. gossypinus*) were positive for borrelial IgG antibodies; neither rice rats (*Oryzomys palustris*) nor Eastern harvest mice (*Reithrodontomys humilis*) were positive for borrelial IgG antibodies. Using Western blot, neither cotton rats or cotton mice were positive; 10% of woodland voles, 7% of golden mice, and 6% of white-footed mice were positive using the presence of 31 or 34 kDa bands present with other bands (15, 21, 26, 39, 41, 66, and 83 kDa). In rodents, unlike borrelial antibodies in raccoons from Shelby county, a higher ELISA titer was not associated with a positive Western blot. In addition, the distribution of rodents with borrelial antibodies was independent of raccoons with borrelial antibodies, indicating that interaction between these mammal groups may not be a factor in animals becoming infected. The immunologic evidence reported in this study and the recent isolations of *Borrelia burgdorferi* in the southern United States indicates that further research on Lyme borreliosis in Tennessee is necessary, including isolation of the spirochete.

Key words: *Borrelia burgdorferi*, Lyme disease, antibodies, enzyme-linked immunosorbent assay, Western blot, Cricetidae, rodent

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INTRODUCTION

Borrelia burgdorferi, the causative agent of Lyme disease, has been isolated from many wild mammals. *B. burgdorferi* was first isolated from white-footed mice and raccoons.[1,2] Since then, a number of different wild mammals, birds, and ticks have been tested for presence of the spirochete or antibodies to *B. burgdorferi* using enzyme-linked immunosorbent assays (ELISA) and immunofluorescent assays (IFA).[3-6] Magnarelli, Oliver, Hutcheson, and Anderson found ELISA to be more sensitive than IFA, and more suitable for testing numerous serum samples for antibodies to *B. burgdorferi*.[7] In the southern United States, ELISA have been used to detect borrelial antibodies in sera

from white-tailed deer *(Odocoileus virginianus)*, cotton mice *(Peromyscus gossypinus)*, white-footed mice *(Peromyscus leucopus)*, and raccoons *(Procyon lotor)*.[8-13] Western blot analysis has been used to detect borrelial antibodies and confirm ELISA tests in humans and other mammals.[7,9,14-15] No reports of borrelial antibodies in rodents have been made in Tennessee.

The Memphis and Shelby County Health Department began a tick research project in 1990 to ascertain the risk of Lyme disease to the human population in western Tennessee. One aspect of this project was to determine if rodents have antibodies to *B. burgdorferi*. Sera from rodents were tested for antibodies to *B. burgdorferi* using the ELISA and Western blot methods to determine if the potential for Lyme disease is present in Tennessee.

METHODS

Study sites and sampling

A total of 49 one-acre sites were selected for trapping rodents from June 1990 through August 1991. Within each site, 40 live traps (Sherman Trap Company) were placed in a line transect or grid, depending on topography. Captured rodents were euthanized with rompun, examined for ticks, and blood was collected by heart puncture. Serum samples were stored at -70°C until they were tested.

Serologic tests

The ELISA method was used with modifications.[8,9] Microwell plates adsorbed with whole cell sonicated (WCS) *B. burgdorferi* strain B-31 were provided by Zeus Laboratories, Raritan, NJ. Goat-anti white-footed mouse (*P. leucopus*) IgG-peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, Md) was absorbed with diluent containing *Treponema phagedenis* (MHA-TP, Miles Laboratories, Elkhart, Ind) to reduce nonspecific binding (1:100 dilution). Goat-anti white-footed mouse IgG conjugate detected IgG of cricetid rodents but was not effective in detecting IgG from murid or sciurid rodents. Other researchers should note that the goat-anti white-footed mouse conjugate cross-reacted with the WCS, but with absorption with MHA-TP, all cross-reactivity ceased.

Dr Louis Magnarelli (Connecticut Agricultural Research Station) graciously provided sera from two positive and one negative white-footed mouse control. Sera were screened at a dilution (in PBS-Tween) of 1:160, also used by other researchers.[16] If positive, sera were serially diluted to an end titer or estimated using a standard curve based on positive controls. The substrate used was o-phenylenediamine (1,2-benzenediamine). Nine sera from white-footed mice testing negative by Western blot were used in addition to the negative control provided by Dr Magnarelli. A net optical density (OD) ≥ 0.05 was considered positive for the diluted sera from rodents based on the mean OD of negative controls (0.02) plus three standard deviations (0.03). All sera testing positive by ELISA were then tested by Western blot analysis.

Western blot analysis was conducted to confirm all serum samples that tested positive using ELISA. Western blot analysis was conducted following Kollars, Ourth, and Lockey,[9] using test strips from the Lyme Disease MARBLOT strip test system using *B. burgdorferi* strain B-31 (March Diagnostics Inc, Carlsbad, Calif). A serum sample was considered positive using the Mardx criteria from 1993 (31 and 34 kDa bands occurring together or one of these two bands occurring with at least one of the following bands: 25, 39, 41, or 83). Sera from the same positive and negative controls used in the ELISA tests were used as controls for Western blots. Statistical comparisons between the percent of rodents positive by ELISA and Western blot were conducted using Chi-square analysis and stepwise Bonferroni adjustment. Chi-square and Fisher's exact test for small sample size were used to test whether the distribution of rodents positive by both ELISA and Western blot were independent of the distribution of raccoons found positive by both tests.[9]

RESULTS

Sera from 37 of 170 individuals from seven cricetid rodent species (22%) tested positive for borrelial antibodies using ELISA. Titers ranged from 1:160 to 1:2560 for borrelial antibodies. Using the ELISA, none of 17 rice rats *(Oryzomys palustris)*, neither of two eastern harvest mice *(Reithrodontomys humilis)*, 9% (4/47) of cotton rats *(Sigmodon hispidus)*, 19% (4/21) of woodland voles *(Microtus pinetorum)*, 31% (21/68) of white-footed mice *(P. leucopus)*, 57% (8/14) of golden mice *(Ochrotomys nuttalli)*, and 100% (1/1) of cotton mice (*P. gossypinus*) were positive for borrelial IgG antibodies. No cotton rats or cotton mice were positive; 10% of woodland voles, 7% of golden mice, and 6% of white-footed mice were positive using the presence of 31 or 34 kDa band in individuals positive by ELISA tested by Western blot analysis. Seven rodents tested positive for borrelial antibodies by Western blot analysis, representing 4% of total rodents tested (137) or 19% of positive ELISAs (37) (Table). The 41 kDa band was the most commonly found band using positive ELISA but negative Western blot serum data. No significant differences between the percentage positive by both tests with titers of 160 through 2560 ($P \geq 0.05$) were found. Three of five areas of positive Western blot rodents co-occurred with three of six positive Western blot sites of raccoons. The distribution of rodents positive by both tests was independent of raccoons positive by both tests ($\chi^2=0.11$, Fisher's $P \geq 0.05$). A map of Shelby County showing sites from

Table
Cricetid Rodents With Positive ELISAs and Negative Western Blots and Individual Rodents Positive by Both ELISA and Western Blot† for Antibodies to* **Borrelia burgdorferi**

	Titers	Without bands	15	18	21	31	34	39	41	60	66	75	83
Negative rodents	160-2560	5	3	1	2	-	-	5	22	16	18	8	7
Positive rodents with identification number													
Microtus pientorum (woodland vole)													
820	160		-	-	-	-	x	x	x	x	x	x	x
856	160		-	-	-	x	-	-	x	x	x	-	x
Ochrotomys nuttalli (golden mouse)													
622	320		-	-	-	-	x	x	x	x	x	x	x
Peromyscus leucopus (white-footed mouse)													
76	1280		-	-	-	x	-	-	x	-	-	-	-
280	1280		-	-	-	-	x	x	x	-	-	-	-
481	160		x	-	-	x	x	x	x	-	-	-	-
740	640		-	-	-	x	-	-	x	-	-	x	-

**Individuals may have more than one antibody shown.*
†Individual bands shown.

which rodents tested positive using Western blot assay are shown in the Figure.

DISCUSSION

The ELISA results in this study (22% positive) were similar to results in other Eastern states. White-footed mice and cotton mice from other states in the southern United States were positive at 36% and 27%, respectively.[7] Sera positive by the ELISA were confirmed using commercially available Western blot strips. Cross-reactivity of antibodies can occur in human disease; this also may be true for wild mammals.[14] Serologic testing for *B. burgdorferi* may give false positive results due to shared antigens with other spirochetes.[17-19] Although rare, cross-reactivity to 31 or 34 kDa bands (OspA and OspB, respectively) by serum antibodies to other diseases can occur in humans. Reactivity to both 31 and 34 kDa bands, however, only occurred in positive control patients.[20] According to Hilton, Devoti, and Sood,[21] the presence of 5 of the following 12 bands is considered a positive Western blot: 18, 21, 28, 30, 31, 34, 39, 41, 45, 58, 66, and 93 kDa. The 18, 21, 31, 34, 41, and 66 kDa bands were present in some rodents (Table); however, because of the controversial nature of Lyme disease in the southeastern United States and the lack of an isolate of *B. burgdorferi* in Tennessee, we chose a conservative approach.

The percentage of rodents having borrelial antibodies was reduced (ELISA vs Western blot results) from 22% to 4% (Table). Few reports of Western blots of wild captured mammals have been reported. In deer, the presence of antibodies to 31 and 34 kDa proteins in Minnesota was 2% in experimentally inoculated deer.[13] In Tennessee, 47% of raccoons tested positive by ELISA and 12% by Western blot using the 31 and 34 kDa criteria.[9]

Although conservative criteria may exclude detecting antibodies to non-*Borrelia* species, antibodies against another or multiple *Borrelia* species may have been detected as indicated by the presence of other *B. burgdorferi* diagnostic bands.[21] Apparent geographic variation and heterogeneity of *Borrelia* species exist in the United States. A *Borrelia* species was isolated from dogs in Florida[22] and a new species (*B. andersonii*) has been described from rabbits collected in the eastern United States.[23] In addition, phenotypic variation has been shown to occur in *Borrelia* isolates from Illinois[24] and Missouri (personal observation, T.M.K.); mixed infection of different *Borrelia* species has been shown to occur in wild mammals[25] and ticks (personal observation, T.M.K.). Isolates of *B. burgdorferi* from cotton mice and cotton rats have been made in the southern United States[26]: 1 of 45 *I. scapularis* was PCR positive using flagellin primer (personal observation, T.M.K.),[27] and *B. burgdorferi* may be endemic in some southeastern states, unlike previous assumptions.[28]

The use of local strains of *B. burgdorferi* does not appear to be required for optimal sensitivity of ELISA[29];

however, differences in Western blots can occur when different strains are used for human sera.[30] Different strains of *B. burgdorferi* also show the same ELISA (no variance) and Western blot (with variance) pattern in laboratory-inoculated white-footed mice.[31]

Positive titers were found in rodents from rural areas of Shelby County and urban areas within the city of Memphis and indicate that rodents are infected with *B. burgdorferi* or possibly some other *Borrelia* species. Similar reductions in the number of animals positive by ELISA and positive Western blots occurred in rodents and raccoons in Shelby County. The data indicate that in rodents, unlike borrelial antibodies in raccoons from Shelby County, a higher ELISA titer was not associated with a positive Western blot. In addition, the distribution of raccoons with borrelial antibodies is independent of rodents with borrelial antibodies. This indicates that interaction between raccoons and cricetid rodents is not necessary for animals to become infected; ie, ticks from mice are not necessarily needed to infect raccoons.

Human cases of Lyme borreliosis have been reported in Tennessee.[32] Serologic testing of wild mammals such as raccoons and rodents can provide important information in surveillance programs[7] and may indicate areas of increased risk for Lyme disease in Tennessee. We suggest that *B. burgdorferi* or another *Borrelia* spp. infects raccoons and rodents in western Tennessee. The occurrence of *B. burgdorferi* in the southeastern United States has been documented and isolates have been made from southeastern Missouri about 150 miles north of Memphis.[33] Although tick species shown to be vectors of *B. burgdorferi* in other states[34-38] have been collected in Shelby County,[39-42] the isolation of *B. burgdorferi* from ticks or wild mammals is necessary to confirm the presence of this spirochete in Tennessee and is the focus of ongoing studies.

Ixodes dentatus commonly is found on rabbits in Shelby County and also has been found on a white-footed mouse in the county.[43] This tick species probably plays an important role in maintaining *B. burgdorferi* or other *Borrelia* species in an enzootic cycle in rabbits in nearby southeastern Missouri and other areas of the eastern United States.[24,33,44] Recent evidence of a possibly new *Borrelia* species from the lone star tick *(Amblyomma americanum)*[45] and the variability of *B. burgdorferi* isolated from southeastern Missouri[44] indicate the wide variety of *Borrelia* species or strains wild mammals are exposed to in the southeastern United States. Based on the serologic evidence, further research of the interactions and ecology among borrelial spirochetes, ticks, and hosts is necessary in Tennessee.

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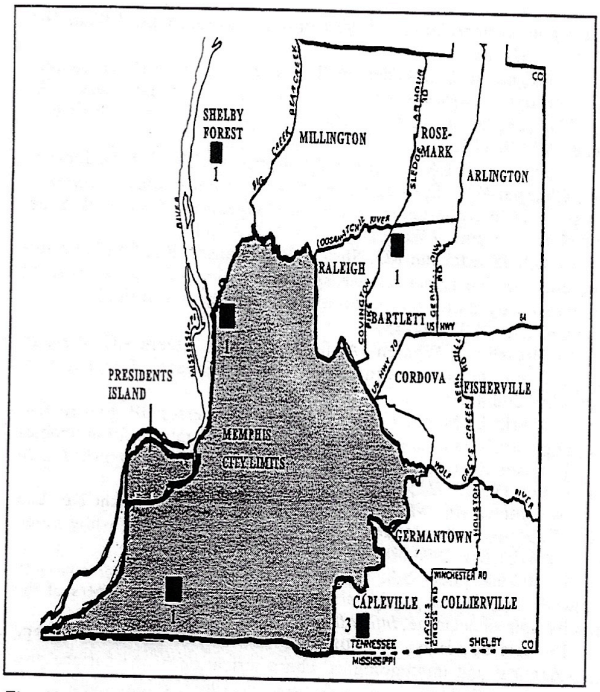

Fig: Number of cricetid rodents in areas of Shelby County, Tenn. that were positive by both enzyme-linked immunosorbent and Western blot assays for antibodies against Borrelia burgdorferi.

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