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ANALYSES OF MAMMALIAN SERA IN ENZYME-LINKED IMMUNOSORBENT ASSAYS WITH DIFFERENT STRAINS OF *BORRELIA BURGDORFERI* SENSU LATO

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ABSTRACT: Blood samples were collected from cottontail rabbits (*Sylvilagus floridanus*), raccoons (*Procyon lotor*), white-footed mice (*Peromyscus leucopus*), and white-tailed deer (*Odocoileus virginianus*) between 1977 and 1991 in southern Connecticut and New York State (USA) and were tested for antibodies against eight strains of *Borrelia burgdorferi* sensu lato in enzyme-linked immunosorbent assays. Among these spirochetes were six strains of *B. burgdorferi* sensu stricto, one strain of *B. garinii* (=IP90) and a strain (IPF) in group VS461. Sera from each study group reacted positively to all strains having origins in North America and Eurasia. Assay sensitivities normally ranged between 85% and 100% for all study groups. The lowest sensitivity (66%) was noted when mouse sera were tested with *B. garinii*, an isolate from *Ixodes persulcatus* in the former Soviet Union. Differences in serum reactivity to various strains were noted for all study groups, but because of multiple shared antigens among the closely related spirochetes tested, the selection of a particular North American strain of *B. burgdorferi* sensu stricto did not appear to be a critical factor for optimal assay performance. Locally obtained strains of this bacterium are preferred as coating antigens for serologic testing because of their availability.

Key words: *Borrelia burgdorferi*, *Borrelia garinii*, Lyme borreliosis, enzyme-linked immunosorbent assay, cottontail rabbits, *Sylvilagus floridanus*, white-tailed deer, *Odocoileus virginianus*, white-footed mice, *Peromyscus leucopus*, raccoons, *Procyon lotor*.

INTRODUCTION

Borrelia burgdorferi infects mammals in forested areas of northeastern and upper midwestern United States (Godsey et al., 1987). The occurrence of human cases is well documented there, in other regions of the United States, and in Eurasia (Wilske et al., 1988; Fukunaga et al., 1993). Although clinical evidence of *B. burgdorferi* infection in domesticated and wild animals usually is lacking, lagomorphs, rodents and other mammals that live near or in tick-infested woodlands develop antibodies to this bacterium (Magnarelli et al., 1984a, 1990, 1991, 1992). Isolations of *B. burgdorferi* sensu lato from forest-dwelling ticks and mammals have confirmed the presence of this spirochetosis (Anderson et al., 1983; Loken et al., 1985). The white-footed mouse, *Peromyscus leucopus*, is the chief reservoir of *B. burgdorferi* sensu stricto in the northeast and upper Midwest of the United States (Levine et al., 1985). Moreover, antibody responses to this bacterium have been investigated in experimentally infected mice (Schwan et al., 1989). Other rodents, such as eastern chipmunks (*Tamias striatus*) also may serve to infect immature ticks with *B. burgdorferi* (McLean et al., 1993), albeit to a lesser extent than white-footed mice.

In attempts to define the geographic distribution of Lyme borreliosis and to identify ecologic factors that increase risk of human exposure to the etiologic agent, serologic tests have been used to determine prevalence of seropositive animals. The B31 Shelter Island, New York (USA) strain (ATCC 35210) of *B. burgdorferi* sensu stricto often is included as antigen in indirect fluorescent antibody staining and enzyme-linked immunosorbent assays (ELISA) because of its availability. In other research laboratories, closely related strains of this spirochete have been selected for use as an antigen. It is unclear, however, if certain strains of *B. burgdorferi*

sensu lato are more advantageous than others are when used as coating antigen in laboratory assays for wildlife. Our purpose was to test sera from lagomorphs, rodents, and other mammals with strains of *B. burgdorferi* sensu lato used earlier (Magnarelli et al., 1994) in an ELISA and to determine if a locally acquired antigen is necessary for optimal assay performance.

MATERIALS AND METHODS

Serum samples, previously shown to have antibodies to *B. burgdorferi* (strain 2591), were chosen to represent the following animals in comparative analyses: cottontail rabbits (*Sylvilagus floridanus*), raccoons (*Procyon lotor*), white-footed mice, and white-tailed deer (*Odocoileus virginianus*). Blood samples were obtained during the period 1977 to 1991 from animals that lived in areas of southern Connecticut (41°28'N, 72°20'W) and in the lower Hudson River Valley region (41°47'N, 73°41'W) of New York State (USA). *Ixodes scapularis*, the chief vector of *B. burgdorferi* sensu stricto, is abundant at these sites. Sera were stored at −60 C at The Connecticut Agricultural Experiment Station, New Haven, Connecticut. There was minimal freezing and thawing of serum samples. With reports of human cases of Lyme borreliosis in Connecticut and New York State (Cartter et al., 1989; Tsai et al., 1989) and isolations of *B. burgdorferi* from ticks and mammals (Anderson et al., 1985, 1989), there is strong evidence of numerous foci of this spirochetosis. Descriptions of study sites, details on serum collections, and results of assay sensitivities and specificities have been reported by Magnarelli et al. (1984b, 1991, 1992).

All serum specimens were originally analyzed in an ELISA with strain 2591 of *B. burgdorferi*, hereafter considered the reference antigen, and subsequently were tested with seven other strains of this bacterium isolated in North America and Eurasia. Included were six isolates of *B. burgdorferi* sensu stricto, one strain of *B. garinii* (IP90), and a *Borrelia* isolate (IPF) in group VS461 (Baranton et al., 1992; Marconi et al., 1992). Some isolates in group VS461 have been classified as *B. afzelii* (Belfaiza et al., 1993). The sources of all eight strains and details on methods of preparing antigens used in the present study have been reported (Anderson et al., 1988; Magnarelli et al., 1994). The hosts and localities from which these isolates were obtained and approximate numbers of passages in cultures are summarized (Table 1). Briefly, the spirochetes were grown in fortified Barbour-Stoenner-Kelly II medium (Anderson and Magnarelli, 1992). Standardized preparations of washed whole cells of all strains were used as antigens in an ELISA.

Polyvalent assays were used to detect serum antibodies to Lyme borreliosis spirochetes and to determine titration end points. Details on materials and methods used in ELISA's for quantitation of total immunoglobulins and on procedures for determining positive results have been described by Magnarelli et al. (1990, 1991, 1992). In short, antigens were standardized as in previous work (Magnarelli et al., 1994) to 3 to 5 μg of protein per ml before coating to the solid phase. Following blocking procedures, serial dilutions of sera were screened with different strains of *B. burgdorferi* sensu lato in parallel tests. Polyvalent affinity-purified, peroxidase-conjugated goat antibodies (e.g., heavy and light chain specific goat anti-*P. leucopus*, etc.) were used followed by plate washings and introduction of 2,2'azino-di-(3-ethyl-benzthiazoline sulfonate) substrate. Absorbance values (optical densities) were recorded at 414 nm by using a microplate photometer. All tests included controls for positive and negative sera, diluents, conjugates, and buffer solutions. In addition to fresh antigen preparations, new lots of conjugated antibodies were standardized before use to ensure consistent assay performance. Tests on reproducibility of antibody titers were included to assess variability of results over a period of 2.5 yr.

For each series of serum samples tested with different strains of antigen, geometric mean antibody titers were calculated. The Wilcoxon signed rank test (Ostle, 1963) was used to determine whether antibody titers recorded for an ELISA with selected strains of *B. burgdorferi* sensu lato were significantly greater or lower than those recorded when reference antigen 2591 was used. Statistical analyses were based on the absolute value of the smaller of the two sums of ranks (T). Critical regions were established based on the number of non-zero difference values computed for paired samples at the $P > 0.01$ level of significance.

RESULTS

Sera from each study group reacted with all strains of *B. burgdorferi* sensu lato in antibody assays. In general, similar assay sensitivities were recorded (Table 2) when percent positive values were compared to those of analyses with the reference antigen strain 2591. Relatively consistent results (sensitivities ≥80%) were obtained when sera from cottontail rabbits, raccoons, white-footed mice, and white-tailed

TABLE 1. Sources of eight strains of *B. burgdorferi* sensu lato antigens used in comparative serological studies.

Antigen strains	Hosts	Source localities	Antigen preparations: Number of passages in cultures
2591[a]	White-footed mouse	E. Haddam, Connecticut (USA)	>200
B31	*Ixodes scapularis*	Shelter Island, New York (USA)	>30
25015	*I. scapularis*	Millbrook, New York	<15
231	*I. scapularis*	Long Point, Ontario, Canada	<15
NCH-1	Human skin	Wisconsin (USA)	<15
33203	*I. pacificus*	Placerville, California (USA)	<15
IPF	*I. persulcatus*	Japan	<15
IP90	*I. persulcatus*	Khabarovsk Territory[b]	<15

[a] Strain used extensively at The Connecticut Agricultural Experiment Station in serologic tests for antibodies and designated as the reference antigen in comparative analyses.
[b] Former Soviet Union.

deer were analyzed. Assay sensitivities for mouse sera, however, were most variable; percent positive values ranged from 66% to 100%. There was noticeably lower assay sensitivity in analyses of mouse sera with strain IP90 (Table 2).

Antibody titers were highly variable. For each animal group, positive titers usually ranged between dilutions of 1:160 and 1:5,120, regardless of the antigen strain used. The highest titers (1:40,960) were recorded for raccoon sera tested with strains 2591, NCH-1, and B31 and for rabbit sera tested with strains 231 and NCH-1. Titration end points were compared for antibody assays with strains 2591 or IPF used as antigens. The respective origins of these spirochetes, Connecticut and Japan, represented the greatest geographic distance for all of the strains tested. Frequency distributions for antibody titers obtained in parallel tests were similar (Table 3). Of the 214 tests performed, most (66%) antibody titers ranged between 1:640 and 1:5,120. Titration end points of 1:640 and 1:1,280 were most frequent (40%). High concentrations of antibodies (≥1:10,240) were detected in sera from all animals except white-tailed deer.

Geometric mean antibody titers varied greatly. Values ranged from a low of 208 for white-footed mouse sera tested with strain IP90 to a high of 2,252 for cottontail rabbit sera analyzed with strain NCH-1 (Table 4). Mean antibody titers for rabbit, raccoon, and mouse sera were relatively higher than those recorded for deer in all tests except with strain IP90. With this strain, the geometric mean titer for deer sera exceeded that of mouse sera but was

TABLE 2. Reactivity of animal sera to various strains of whole cell *Borrelia burgdorferi* sensu lato in a polyvalent enzyme-linked immunosorbent assay and percent seropositivity.

Study groups	*Borrelia burgdorferi* strains[a] 2591	B31	25015	231	NCH-1	33203	IPF	IP90
Cottontail rabbits	27 (100)[b]	27 (100)	27 (93)	27 (96)	27 (100)	25 (80)	27 (96)	27 (93)
Raccoons	24 (100)	24 (100)	24 (88)	24 (79)	24 (92)	24 (92)	24 (100)	24 (96)
White-footed mice	30 (100)	29 (97)	30 (80)	30 (93)	29 (97)	24 (100)	29 (86)	29 (66)
White-tailed deer	27 (100)	26 (85)	27 (89)	27 (85)	26 (89)	25 (100)	26 (89)	26 (85)

[a] Sources of strains given in Table 1.
[b] Number of sera tested (percent with antibodies present).

TABLE 3. Frequency distributions of antibody titers for animal sera in polyvalent enzyme-linked immunosorbent assay with strains 2591 and IPF of *Borrelia burgdorferi* senu lato.

Study groups	Strain	Number of sera with reciprocal antibody titers of					
		<160	160–320	640–1,280	2,560–5,120	10,240–20,480	40,960
Cottontail rabbits	2591[a]	0	2	14	10	1	0
	IPF[b]	1	3	12	8	3	0
Raccoons	2591	0	3	8	9	2	2
	IPF	0	3	10	8	3	0
White-footed mice	2591	0	2	13	11	4	0
	IPF	5	10	10	3	1	0
White-tailed deer	2591	0	15	9	3	0	0
	IPF	3	10	10	3	0	0

[a] Isolate from a white-footed mouse captured in East Haddam, Connecticut.
[b] Isolate from *Ixodes persulcatus* collected in Japan.

at least three-fold less than the mean titers recorded for rabbit and raccoon sera. Paralleling results of assay sensitivity, lower geometric mean antibody titers were recorded for mouse sera analyzed with strains IPF and IP90. Uniformly low geometric mean antibody titers (range = 312 to 622) were recorded for deer sera tested with all eight strains of antigens.

Statistical analyses were conducted to determine if antibody titers in an ELISA with strain 2591 were significantly greater or lower than those recorded in assays with other strains. Depending on the antigen used, results were mixed (Table 5) for cottontail rabbits. Antibody titers for these study groups were significantly greater in assays with strains 231 and NCH-1 and lower when strain 25015 was used. In analyses of raccoon and mouse sera, antibody titers were either significantly lower than, or statistically similar to, those recorded in assays with strain 2591. In analyses of ranks of paired data for deer sera, there were no significant differences in results, regardless of the antigen used.

Forty serum samples were retested against the eight strains of Lyme borreliosis spirochetes (total trials = 320) to assess reproducibility of titration end points. When results of all study groups were combined (Table 6), antibody titers for the majority of specimens (66% of 320) were the same or differed by two-fold. Titers varied by four-fold or less in 270 (84%) replicates. Titers for the remaining 50 trials changed by eight-fold or greater.

DISCUSSION

Sera from each study group reacted positively in an ELISA, regardless of the strain of Lyme borreliosis spirochete used. With comparable assay sensitivities, it is clear that there are multiple shared antigens

TABLE 4. Geometric mean antibody titers for animal sera in polyvalent enzyme-linked immunosorbent assay with different strains of *Borrelia burgdorferi* sensu lato.

Study groups	Geometric mean antibody titers and strains[a] of *B. burgdorferi*							
	2591	B31	25015	231	NCH-1	33203	IPF	IP90
Cottontail rabbits	1,532	1,493	827	2,139	2,252	997	1,418	1,248
Raccoons	1,974	1,810	783	698	1,709	1,208	1,759	1,174
White-footed mice	1,810	916	463	1,896	1,626	1,356	397	208
White-tailed deer	483	329	376	312	503	622	386	329

[a] Sources of strains given in Table 1.

TABLE 5. T values and number of non-zero difference values for Wilcoxon signed rank tests using strain 2591 as the reference antigen in parallel enzyme-linked immunosorbent assay.

Study groups	Strains of *Borrelia burgdorferi*[a]						
	B31	25015	231	NCH-1	33203	IPF	IP90
Cottontail rabbits	28.5[b]	0 (<)[c]	8.3 (>)	15.6 (>)	48.3	31.6	49.9
Raccoons	52.6	23.3 (<)	11.2 (<)	63.7	38.5	47.3	42
White-footed mice	35.8 (<)	2 (<)	82.7	82.8	24	28.5 (<)	2 (<)
White-tailed deer	36.4	25.6	18.3	46.2	42.8	63	35.9

[a] Sources of strains given in Table 1.

[b] T = absolute value of the smaller of the two sums of ranks. Number of non-zero values for all paired tests ranged between 11 and 28.

[c] Sum of ranks for antibody titers were significantly greater (>) or lower (<) than that of an ELISA with strain 2591 used as antigen at $P > 0.01$ level of significance.

among all eight strains tested and that, in general, there was no apparent disadvantage in using spirochetes that had been subcultured numerous times. However, results of lower assay sensitivities for mouse sera tested with strains IPF and IP90, compared to North American isolates, might be evidence for marked differences in antigenic compositions. Antigenic diversity exists among strains of *B. burgdorferi* sensu lato (Anderson et al., 1988, 1989; Wilske et al., 1988; Baranton et al., 1992; Fukunaga et al., 1993; Park et al., 1993). Moreover, even though there appears to be many shared antigens among *B. burgdorferi* sensu lato, mammalian hosts may not immunologically recognize multiple common epitopes in the same manner. For mice in particular, an antigen of North American origin is preferred for serologic tests because of availability, greater sensitivity, and less variable test results.

Concentrations of antibodies in sera from all animal groups varied greatly. Histories of tick bites and initial times of exposure to *B. burgdorferi* are unknown in most instances. Consequently, it is unclear if titers represented current or past infections. Blood samples probably were acquired from animals in different stages of infection. Further, tick infection prevalences vary in different sites (Schulze et al., 1986; Stafford and Magnarelli, 1993), and the degree of host exposure to *B. burgdorferi* will depend, partly, on frequency of animal contact with infected ticks in or near forests. Therefore, it is not unusual to record a wide range of antibody titers for a group of sera. Aside from the noted differences in assay sensitivity for mice, the similar frequency distributions of antibody titers recorded for assays with two strains of Lyme borreliosis spirochetes collected in Connecticut and Japan and results of replicated trials are evidence that there is a high degree of relatedness between these bacteria and that titration end points were reproducible.

TABLE 6. Reanalyses of serum specimens and frequency distributions for reproducibility of antibody titers in an enzyme-linked immunosorbent assay with eight whole cell strains of *Borrelia burgdorferi* sensu lato.

Study groups	Total sera retested	Total replicates	Differences in antibody titers[a]				
			None	2-fold	4-fold	8-fold	>8-fold
Cottontail rabbits	12	96	35	32	17	1	11
Raccoons	12	96	31	27	22	3	13
White-footed mice	5	40	18	10	8	2	2
White-tailed deer	11	88	25	32	13	10	8

[a] Reproducibility trials were conducted within 2.5 years after initial assays were performed. Different lots of the same antigen strain were standardized along with other reagents in ELISA before use.

In parallel tests with strain 2591 of *B. burgdorferi* being used as a reference antigen, antibody titers in cottontail rabbit sera were consistently higher when strains 231 and NCH-1 were included in analyses. These results were consistent with those recorded for human Lyme borreliosis sera (Magnarelli et al., 1994). We recognize that elevated concentrations of antibodies might be due in some instances to greater reactivity of sera to low passage isolates used in an ELISA. Alternatively, there may be antigens associated with some strains that are lacking in others. Distinct differences in protein profiles of some strains of *B. burgdorferi* sensu lato have been documented by polyacrylamide gel electrophoresis or DNA analyses (Wilske et al., 1988; Belfaiza et al., 1993). Further, nonprotein antigenic components, such as lipooligosaccharides, also may be seroreactive (Wheeler et al., 1993). The antigenic compositions of closely related strains of *B. burgdorferi* sensu lato and specific immune responses of hosts to these components need further study.

In general, the selection of a particular North American whole cell *B. burgdorferi* strain for analyses did not appear to be a critical factor. Differences in reactivity of sera with some strains were noted, but for routine screening of specimens in epizootiologic studies of Lyme borreliosis in northeastern or northcentral United States, use of most strains of *B. burgdorferi* isolated in those respective regions should be suitable for serologic testing. Similar conclusions have been made following the analyses of human sera with autologous and heterologous isolates of *B. burgdorferi* (Berger et al., 1988; Karlsson, 1991; Magnarelli et al., 1994). However, to strengthen epizootiologic and epidemiologic studies, efforts should still be made to confirm *B. burgdorferi* infections by culturing these spirochetes from ticks and vertebrate hosts.

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LITERATURE CITED

ANDERSON, J. F., AND L. A. MAGNARELLI. 1992. Epizootiology of Lyme disease and methods of cultivating *Borrelia burgdorferi*. Annals of the New York Academy of Sciences 653: 52–63.

———, ———, W. BURGDORFER, AND A. G. BARBOUR. 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. American Journal of Tropical Medicine and Hygiene 32: 818–824.

———, R. C. JOHNSON, L. A. MAGNARELLI, AND F. W. HYDE. 1985. Identification of endemic foci of Lyme disease: Isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). Journal of Clinical Microbiology 22: 36–38.

———, L. A. MAGNARELLI, AND J. B. MCANINCH. 1988. New *Borrelia burgdorferi* antigenic variant isolated from *Ixodes dammini* from upstate New York. Journal of Clinical Microbiology 26: 2209–2212.

———, ———, R. B. LEFEBVRE, T. G. ANDREADIS, J. B. MCANINCH, G.-C. PERNG, AND R. C. JOHNSON. 1989. Antigenically variable *Borrelia burgdorferi* isolated from cottontail rabbits and *Ixodes dentatus* in rural and urban areas. Journal of Clinical Microbiology 27: 13–20.

BARANTON, G., D. POSTIC, I. SAINT GIRONS, P. BOERLIN, J.-C. PIFFARETTI, M. ASSOUS, AND P. A. D. GRIMONT. 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. International Journal of Systematic Bacteriology 42: 378–383.

BELFAIZA, J., D. POSTIC, E. BELLENGER, G. BARANTON, AND I. SAINT GIRONS. 1993. Genomic fingerprinting of *Borrelia burgdorferi* sensu lato by pulsed-field gel electrophoresis. Journal of Clinical Microbiology 31: 2873–2877.

BERGER, B. W., A. B. MACDONALD, AND J. L. BENACH. 1988. Use of an autologous antigen in the serologic testing of patients with erythema migrans of Lyme disease. Journal of the American Academy of Dermatology 18: 1243–1246.

CARTTER, M. L., P. MSHAR, AND J. L. HADLER. 1989. The epidemiology of Lyme disease in Connecticut. Connecticut Medicine 53: 320–323.

FUKUNAGA, M., M. SOHNAKA, Y. TAKAHASHI, M. NAKAO, AND K. MIYAMOTO. 1993. Antigenic and genetic characterization of *Borrelia* species isolated from *Ixodes persulcatus* in Hokkaido, Japan. Journal of Clinical Microbiology 31: 1388–1391.

GODSEY, M. S., JR., T. E. AMUNDSON, E. C. BURGESS, W. SCHELL, J. P. DAVIS, R. KASLOW, AND R. EDELMAN. 1987. Lyme disease ecology in Wisconsin. Distribution and host preferences of *Ixodes dammini*, and prevalence of antibody to *Borrelia burgdorferi* in small mammals. American Jour-

nal of Tropical Medicine and Hygiene 37: 180–187.

KARLSSON, M. 1991. Antibody responses against autologous and heterologous isolates of *Borrelia burgdorferi* in patients with Lyme neuroborreliosis. European Journal of Clinical Microbiology and Infectious Diseases 10: 742–745.

LEVINE, J. F., M. L. WILSON, AND A. SPIELMAN. 1985. Mice as reservoirs of the Lyme disease spirochete. American Journal of Tropical Medicine and Hygiene 34: 355–360.

LOKEN, K. I., C. WU, R. C. JOHNSON, AND R. F. BEY. 1985. Isolation of the Lyme disease spirochete from mammals in Minnesota. Proceedings of the Society for Experimental Biology and Medicine 179: 300–302.

MAGNARELLI, L. A., J. F. ANDERSON, W. BURGDORFER, AND W. A. CHAPPELL. 1984a. Parasitism by *Ixodes dammini* (Acari:Ixodidae) and antibodies to spirochetes in mammals at Lyme disease foci in Connecticut, USA. Journal of Medical Entomology 21: 52–57.

———, J. M. MEEGAN, J. F. ANDERSON, AND W. A. CHAPPELL. 1984b. Comparison of an indirect fluorescent antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. Journal of Clinical Microbiology 20: 181–184.

———, J. F. ANDERSON, AND J. B. MCANINCH. 1990. Serologic analyses of cottontail rabbits for antibodies to *Borrelia burgdorferi*. Journal of Clinical Microbiology 28: 890–893.

———, J. H. OLIVER, JR., H. J. HUTCHESON, AND J. F. ANDERSON. 1991. Antibodies to *Borrelia burgdorferi* in deer and raccoons. Journal of Wildlife Diseases 27: 562–568.

———, ———, ———, J. L. BOONE, AND J. F. ANDERSON. 1992. Antibodies to *Borrelia burgdorferi* in rodents in eastern and southern United States. Journal of Clinical Microbiology 30: 1449–1452.

———, J. F. ANDERSON, R. C. JOHNSON, R. B. NADELMAN, AND G. P. WORMSER. 1994. A comparison of different strains of *Borrelia burgdorferi* sensu lato used as antigen in enzyme-linked immunosorbent assays. Journal of Clinical Microbiology 32: 1154–1158.

MARCONI, R. T., L. LUBKE, W. HAUGLUM, AND C. F. GARON. 1992. Species-specific identification of and distinction between *Borrelia burgdorferi* genomic groups by using 16S rRNA-directed oligonucleotide probes. Journal of Clinical Microbiology 30: 628–632.

MCLEAN, R. G., S. R. UBICO, AND L. M. COOKSEY. 1993. Experimental infection of the eastern chipmunk (*Tamias striatus*) with the Lyme disease spirochete (*Borrelia burgdorferi*). Journal of Wildlife Diseases 29: 527–532.

OSTLE, B. 1963. Statistics in research. Iowa State University Press, Ames, Iowa, 585 pp.

PARK, K.-H., W.-H. CHANG, AND T. G. SCHWAN. 1993. Identification and characterization of Lyme disease spirochetes, *Borrelia burgdorferi* sensu lato, isolated in Korea. Journal of Clinical Microbiology 31: 1831–1837.

SCHULZE, T. L., M. F. LAKAT, W. E. PARKIN, J. K. SHISLER, D. J. CHARETTE, AND E. M. BOSLER. 1986. Comparison of rates of infection by the Lyme disease spirochete in selected populations of *Ixodes dammini* and *Amblyomma americanum* (Acari: Ixodidae). Zentralblatt für Bakteriologie Mikrobiologie und Hygiene (Series A) 263: 40–44.

SCHWAN, T. G., K. K. KIME, M. E. SCHRUMPF, J. E. COE, AND W. J. SIMPSON. 1989. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). Infection and Immunity 57: 3445–3451.

STAFFORD, K. C. III., AND L. A. MAGNARELLI. 1993. Spatial and temporal patterns of *Ixodes scapularis* (Acari: Ixodidae) in southeastern Connecticut. Journal of Medical Entomology 30: 762–771.

TSAI, T. F., R. E. BAILEY, AND P. S. MOORE. 1989. National surveillance of Lyme disease, 1987–1988. Connecticut Medicine 53: 324–326.

WHEELER, C. M., J. C. GARCIA MONCO, J. L. BENACH, M. G. GOLIGHTLY, G. S. HABICHT, AND A. C. STEERE. 1993. Nonprotein antigens of *Borrelia burgdorferi*. The Journal of Infectious Diseases 167: 651–664.

WILSKE, B., V. PREAC-MURSIC, G. SCHIERZ, R. KUHBECK, A. G. BARBOUR, AND M. KRAMER. 1988. Antigenic variability of *Borrelia burgdorferi*. Annals of the New York Academy of Sciences 539: 126–143.

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