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Serologic Surveillance for the Lyme Disease Spirochete, *Borrelia burgdorferi*, in Minnesota by Using White-Tailed Deer as Sentinel Animals

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To determine the effectiveness of white-tailed deer as sentinel animals in serologic surveillance programs for *Borrelia burgdorferi*, we performed enzyme-linked immunosorbent assay (ELISA) and Western immunoblotting analyses on 467 deer serum samples. The seropositivity rate in the ELISA was 5% for the 150 samples collected at the three sites in which the tick *Ixodes scapularis* was absent. The three sites with established *I. scapularis* populations had a seropositivity rate of 80% for 317 samples. Results were similar for two closely situated sites, one with an established *I. scapularis* population and one without; these sites were only 15 km apart. Rates of seropositivity were significantly higher in yearling and adult deer than in fawns. The mean numbers of bands seen on Western immunoblots were 3.0 for samples negative in the ELISA and 13.8 for samples positive in the ELISA; all of these samples were collected from sites in which *I. scapularis* was established. At sites in which *I. scapularis* was absent, the mean numbers of bands seen were 1.6 for samples negative in the ELISA and 8.2 for samples positive in the ELISA. There were 14 different *B. burgdorferi* antigens that reacted with more than 50% of the ELISA-positive samples from areas with *I. scapularis*. A 19.5-kDa antigen reacted with 94% of the ELISA-positive samples. Reactivity against OspA and OspB was weak and infrequent (2%). Serologic analysis of white-tailed deer sera appears to be an accurate and sensitive surveillance method for determining whether *B. burgdorferi* is present in specific geographic locations.

Lyme borreliosis is the leading arthropod-transmitted bacterial disease in the United States (5). Its etiologic agent is the spirochete *Borrelia burgdorferi* (3, 17, 20), which is transmitted by ticks of the genus *Ixodes*. In the Lyme disease-endemic areas of the north central and northeastern United States, the vector is *Ixodes scapularis* (formerly *I. dammini*) (35). *I. pacificus* is the vector in the western states (1). The hallmark of the disease is an expanding erythematous skin lesion, erythema migrans. Other acute abnormalities may develop in one or more major organ systems. A chronic infection that can persist for many years may develop (41).

A determination of whether the bacterium is endemic in specific geographic areas would provide key information on possible Lyme disease risk. In Minnesota and other parts of the United States, these disease-endemic areas are expanding. At present, human case data, tick identification, and animal studies provide the information for risk assessment in a given area. Most of these methods have certain disadvantages. Human case data may be unreliable because of the ease with which humans travel over large areas, making it difficult to establish with certainty the precise geographic area in which exposure occurred. Current surveillance methods for tick studies, in which ticks are captured, identified, and assessed for spirochete presence, or animal studies, in which small mammals, such as mice, voles, and chipmunks, are live trapped and assessed either by serologic or by culture techniques for *B. burgdorferi*, are very labor-intensive.

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In a previous article (13), we proposed that white-tailed deer (*Odocoileus virginianus*) be used as sentinel animals in serologic surveillance programs. In this study, we confirm the validity of this proposal and demonstrate the qualities of white-tailed deer as sentinel animals. We analyzed by enzyme-linked immunosorbent assay (ELISA) and Western immunoblotting deer serum samples from sites in Minnesota in which *I. scapularis* is absent and in which it has established populations. These samples were collected by hunters during the regular-firearms deer-hunting season.

MATERIALS AND METHODS

Study sites and sampling. Serum samples from white-tailed deer (n = 467) were collected during November and December 1992 at six different sites in Minnesota from both male and female deer varying in age from fawn to adult (Fig. 1). Four sites were located in the seven-county metropolitan area of Minneapolis-St. Paul, and two sites, Wild River State Park and Saint Croix State Park, were situated approximately 30 and 95 km, respectively, north of the metropolitan area in east central Minnesota. Three of the six sites (Carlos Avery Wildlife Management Area [WMA], Wild River State Park, and Saint Croix State Park) were located in areas in which deer tick populations are considered established (4, 12, 13, 33). Samples (n = 425) at five sites were collected by hunters during 2-day deer hunts held to reduce relatively high deer populations at these county or state parks. Samples (n = 42) at the sixth site, Carlos Avery WMA, were collected by hunters over an 8-day period during the regular-firearms deer-hunting season in November. The ages of the

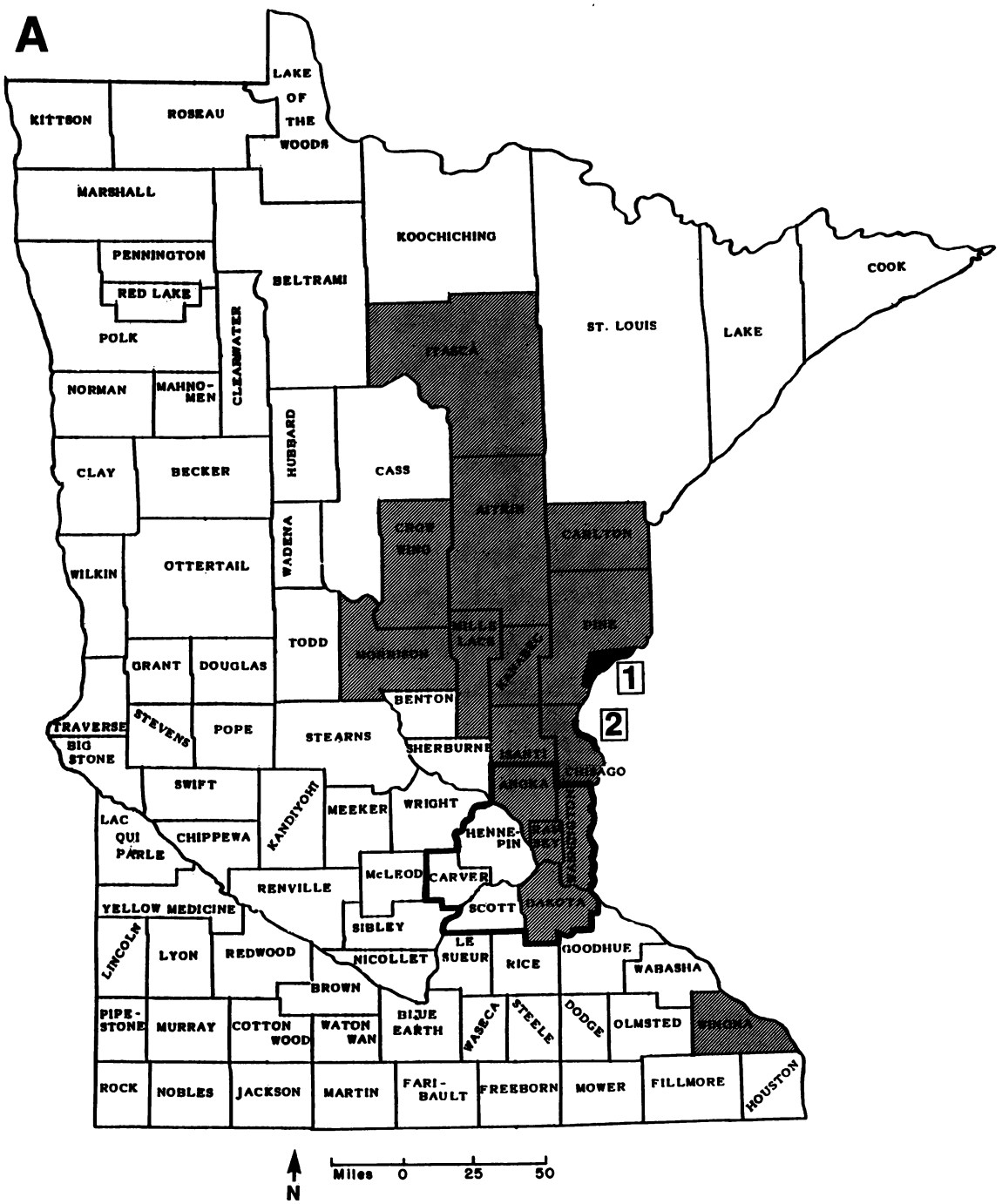

FIG. 1. Maps showing the locations of the six white-tailed deer serum sampling sites used in November and December 1992 and the geographic regions in which populations of *I. scapularis* are considered established. (A) Map of the state of Minnesota. Shaded areas show where *I. scapularis* populations are established by county. *I. scapularis* is not established in the unshaded areas. (B) Map of the seven-county metropolitan area of Minneapolis-St. Paul. *I. scapularis* is established throughout the shaded portion and is rare or absent in the unshaded portion. Deer serum sampling sites: 1, Saint Croix State Park; 2, Wild River State Park; 3, Carlos Avery WMA; 4, Elm Creek Park Reserve; 5, Crow-Hassan Park Reserve; and 6, Lake Rebecca Park Reserve.

deer were determined by trained personnel of the Minnesota Department of Natural Resources according to dental patterns and tooth wear (39).

Control serum specimens. Four groups of pen-raised, tick-free deer were used to provide negative and positive control serum specimens as previously described (13). In brief, one group (n = 4) was immunized with killed, sonicated *B. burgdorferi* NY90-14, a low-passage, virulent isolate from a New York State white-footed mouse, *Peromyscus leucopus*. A second group (n = 2) was immunized with killed *B.*

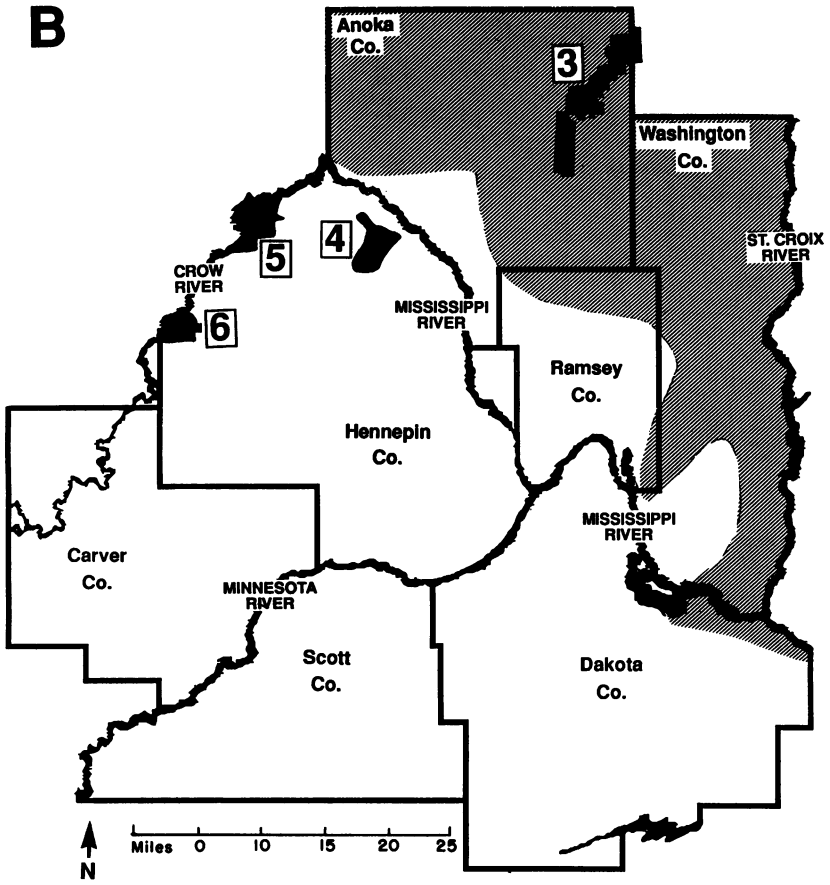

FIG. 1—*Continued.*

burgdorferi B31 (ATCC 35210) originally derived from *I. scapularis* collected in New York State. A third group (n = 1) was immunized with live *B. burgdorferi* SH-2-82, a low-passage, virulent isolate from *I. scapularis* collected in New York State. A fourth group (n = 5) was used as negative controls. Another group (n = 112) of wild deer living in areas devoid of *I. scapularis* (Lake Rebecca Park Reserve and Elm Creek Park Reserve) were used as negative controls to establish the baseline value in the ELISA. Rabbit immune serum was prepared, as previously described (13), with *B. burgdorferi* 297, a spinal fluid isolate from a Connecticut patient. The titer in the indirect immunofluorescence assay was 1:1,024.

Serologic analyses. The procedure for the ELISA has been described elsewhere (11, 13). In brief, the ELISA was carried out with 0.5 μg of washed, sonicated *B. burgdorferi* NY90-14 cell antigen per well in 96-well Immulon 4 plates (Dynatech Laboratories, Chantilly, Va.). Plates were blocked with 3% fetal bovine serum for 60 min to reduce nonspecific reactivity. Deer serum specimens were diluted 1:500 in 0.1 M phosphate-buffered saline (pH 7.4) before 50 μl of sample per well was added. Samples were run in duplicate wells, and each test was repeated. Deer antibodies were detected with horseradish peroxidase-labeled rabbit antideer immunoglobulin G (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) diluted 1:2,000, the substrate, 3,3',5,5'-tetramethylbenzidine (U.S. Biochemical Corp., Cleveland, Ohio) in sodium acetate buffer (pH 6.0), and hydrogen peroxide. Optical density (OD) values were read at 450 nm. The baseline OD value was established by use of the 112 samples from the Lake Rebecca Park Reserve and the Elm Creek Park Reserve, in which *I. scapularis* populations were not established (Fig. 1). Two positive and five negative control samples were included on each ELISA plate as internal quality checks. Samples were considered positive when the OD values were ≥3 standard deviations (SDs) (1 SD = 0.040) above the mean (OD = 0.057) for the baseline samples (n = 112).

Western immunoblotting was carried out as previously described (11, 13). *B. burgdorferi* 297 was the test antigen (150 μg of protein in one large trough well [130 mm] per gel) run on 5 to 20% linear gradient polyacrylamide gels (14 cm by 16 cm by 0.75 mm thick) under reducing conditions with sodium dodecyl sulfate at 25 to 40 mA for approximately 2 h. Electrophoretic transfer to polyvinylidene difluoride paper (Millipore Corp., Bedford, Mass.) was carried out at 1 A for 30 min, after which the paper was cut into strips 2 mm wide. Twenty microliters of serum was diluted 1:250 for testing of each strip. Nonfat dry milk (0.5%) in Tris-buffered saline (0.02 M Tris, 0.5 M NaCl) (pH 7.5) was used to block the nonspecific reactivity of the strips and to dilute serum samples. The presence of reactive deer antibodies was detected with alkaline phosphatase-labeled goat antideer immunoglobulin G (Kirkegaard & Perry Laboratories) diluted 1:500 and the substrate reagents, nitroblue tetrazolium and bromo-chloro-indolylphosphate (Sigma Chemical Co., St. Louis, Mo.). All strips exposed to deer sera were developed at 30 to 32°C for 7 min. Strips of rabbit control

TABLE 1. ELISA results for white-tailed deer serum samples collected in November and December 1992 from areas in Minnesota

Site	Established population of *I. scapularis*	No. of samples tested	No. (%) of samples that were:	
			Positive	Negative
Lake Rebecca Park Reserve	No	40	1 (3)	39 (97)
Crow-Hassan Park Reserve	No	38	2 (5)	36 (95)
Elm Creek Park Reserve	No	72	5 (7)	67 (93)
Carlos Avery WMA	Yes	42	30 (71)	12 (29)
Wild River State Park	Yes	66	53 (80)	13 (20)
Saint Croix State Park	Yes	209	171 (82)	38 (18)

serum (diluted 1:1,000) were developed for 2 min. Rabbit antibodies were detected with alkaline phosphatase-labeled goat antirabbit immunoglobulin G (Cappel/Organon Teknika Corp., West Chester, Pa.) diluted 1:1,000. Five sets of strips evenly distributed across each large original Western blot were reacted with rabbit control serum and fount India ink protein stain (Pelikan AG, Hannover, Germany) to assist in aligning and identifying the antigen bands.

Statistical analysis. For determining statistical differences in the number of samples that were positive in the ELISA versus the number of samples that were negative in the ELISA, χ^2 analysis was used. A P value of ≤ 0.05 was considered significant.

RESULTS

Six sites located in east central Minnesota were used as collection points for 467 white-tailed deer serum samples (Fig. 1); three were in areas that lack established populations of *I. scapularis*, and three were in areas with established *I. scapularis* populations (4, 12, 13, 33). Analysis of these samples in the ELISA revealed that only 8 of the 150 serum samples from the three sites in which *I. scapularis* is absent were positive for anti-*B. burgdorferi* antibodies, yielding a 5% seropositivity rate. The seropositivity rates for the individual sites ranged from 3 to 7% (Table 1). The differences among the rates for the three sites were not significant ($P < 0.50$). ELISA results for the 317 serum samples from the three sites with established *I. scapularis* populations showed that 254 samples were positive, yielding a seropositivity rate of 80%. Seropositivity rates of 71 to 82% were seen for these three sites (Table 1). There were no significant differences among these rates ($P < 0.10$).

At the two state park sites, Wild River and Saint Croix, which have established populations of *I. scapularis* (Fig. 1), data on the age and sex of the deer were obtained for 269 samples (119 from male deer and 150 from female deer). The seropositivity rate for male deer of all ages was 84%, and that for female deer of all ages was 79%. Seropositivity rates for male and female fawns were 67 and 43%, respectively (Table 2). The seropositivity rate for male fawns was significantly different from the rates for male yearlings and adults ($P < 0.005$). Also, a significant difference existed between the seropositivity rate for female fawns and the rates for female yearlings and adults ($P < 0.001$).

A Western immunoblot analysis was performed on totals of 48 and 317 samples from sites in which *I. scapularis* was not established and sites in which *I. scapularis* was established, respectively. These samples included 106 serum specimens that were found negative for anti-*B. burgdorferi* antibodies by the ELISA and 259 that were found positive by the ELISA. The mean numbers of bands per ELISA-negative specimen were similar for sites without *I. scapularis* populations and sites with *I. scapularis* populations (Table 3). For ELISA-positive specimens, the mean numbers of bands tended to be lower for sites lacking *I. scapularis* than for sites in which this tick species was present. Positive deer samples from sites without an established *I. scapularis* population may indicate that deer had immigrated from adjacent endemic areas, that low numbers of *B. burgdorferi*-infected ticks were present, or both. The highest number of bands seen for ELISA-positive samples was 33, and the lowest number of bands was 3. Of 106 ELISA-negative samples, 18 (17%) did not have any bands. Representative Western immunoblots from each of the six sites are shown in Fig. 2. Also shown are results from deer positive and negative control specimens and a rabbit immune control specimen.

TABLE 2. Age and sex analysis of ELISA results for white-tailed deer serum samples collected in November 1992 from Wild River State Park and State Croix State Park[a]

Animals	No. positive/no. tested (%)
Male	
Adult	51/56 (91)
Yearling	27/30 (90)
Fawn	22/33 (67)
Female	
Adult	95/112 (85)
Yearling	14/15 (93)
Fawn	10/23 (43)

[a] Both state parks have established populations of *I. scapularis*.

A total of 58 different antigens were recognized by the 365 samples analyzed by Western immunoblotting. The 254 ELISA-positive samples collected at sites with established *I. scapularis* populations reacted with 56 antigens on Western immunoblots (data not shown). The five ELISA-positive samples from sites in which *I. scapularis* was not established reacted with 22 different antigens. The ELISA-negative samples, 63 from sites in which *I. scapularis* was established

TABLE 3. Western immunoblot analysis of white-tailed deer serum samples collected in November and December 1992 from areas in Minnesota

Site	Negative in the ELISA		Positive in the ELISA	
	No. of samples tested	Mean no. of bands ± SD	No. of samples tested	Mean no. of bands ± SD
I. scapularis absent				
Elm Creek Park Reserve	18	1.6 ± 1.4	4	8.8 ± 2.5
Crow-Hassan Park Reserve	12	1.3 ± 1.4	1	6
Lake Rebecca Park Reserve	13	2.0 ± 2.8		
Total	43	1.6 ± 1.9	5	8.2 ± 2.5
I. scapularis established				
Carlos Avery WMA	12	3.3 ± 2.3	30	15.5 ± 5.2
Wild River State Park	13	2.8 ± 1.9	53	15.7 ± 5.9
Saint Croix State Park	38	2.9 ± 2.2	171	12.9 ± 4.7
Total	63	3.0 ± 2.1	254	13.8 ± 5.2

and 43 from sites in which the tick species was not, reacted with 32 and 27 antigens, respectively. Table 4 lists the 14 antigens that were recognized by more than 50% of the ELISA-positive samples from sites in which *I. scapularis* was present and compares the frequencies with which they reacted with samples that were found negative and positive by the ELISA. The 43-, 41 (flagellin)-, 39.5 (P39)-, 35-, and 19.5-kDa antigens had the highest rates of reactivity, reacting with ≥70% of the positive samples from sites with *I. scapularis*. The 19.5-kDa antigen reacted with 94% of the samples, the highest rate of reactivity observed. For negative samples from sites with *I. scapularis*, the highest rates of reactivity were seen with the 43-, 41-, 35-, 24.5-, and 19.5-kDa antigens. The 19.5-kDa antigen had the highest rate of reactivity (33%). Similar results were seen for negative samples from sites lacking *I. scapularis*. For these samples, the 43-, 41-, and 35-kDa antigens had the three highest rates of reactivity, 17, 26, and 19%, respectively. The 39.5-, 24.5-, and 19.5-kDa antigens were reactive with only 9, 2, and 9% of the samples, respectively. For all samples tested, the frequency and intensity of reactivity with either the 31 (OspA)- or the 34 (OspB)-kDa antigen were very low. Only 7 samples (2%) of the 365 analyzed had detectable antibodies against OspA. All seven of these samples were collected from adult deer. Nine samples (2%) had detectable reactivity with OspB. Five of these samples were from adult (n = 4) or yearling (n = 1) deer, and two were from fawns; the ages of the deer from which the two remaining samples were collected were unknown.

DISCUSSION

In a previous study, we demonstrated that white-tailed deer generate an antibody response against *B. burgdorferi* that is detectable by an ELISA and by Western immunoblotting and that deer are potential sentinel animals for the serologic surveillance of *B. burgdorferi* (13). In the present study, half of our collection sites were located in areas in which populations of *I. scapularis* were established, and the other half were located in areas in which populations of *I. scapularis* were absent (4, 12, 13, 33) (Fig. 1). The absence of *I. scapularis* in Hennepin County was determined previously by extensive small mammal sampling and drag cloth sampling over the entire seven-county metropolitan area (Fig. 1B): 18,714 ticks from 9,217 small mammals and 2,769 drag cloth samples were identified to the species level (13, 33). It is of interest that only 15 km separates Carlos Avery WMA (a site with established *I. scapularis* populations) from Elm Creek Park Reserve (a site without *I. scapularis*). Seropositivity rates at sites with established *I. scapularis* populations were 71 to 82%. At sites without *I. scapularis* populations, seropositivity rates were 3 to 7% (Table 1). These data show that the seropositivity rates reflect the boundaries of *I. scapularis* populations. Data from small mammal trapping studies support this conclusion. Culture specimens from 35 of 135 rodents (26%) captured at Carlos Avery WMA during 1992 were positive for *B. burgdorferi*, while culture specimens from only 2 of 124 rodents (2%) in Elm Creek Park Reserve were positive (19). The relatively sedentary nature of white-tailed deer in east central Minnesota (18, 21, 38, 40) is probably the primary reason for the deer seropositivity rates closely coinciding with *I. scapularis* population boundaries. Deer in this area of Minnesota usually remain within an approximately 2.5-km^2 area during their stay in either their summer or their winter range and usually migrate less than 10 km in their travel back and forth between ranges. However, nonmigratory travel by deer over much greater distances has been reported (38).

Adult and yearling deer of both sexes had higher seropositivity rates than fawns from the same area, indicating that fawns should be excluded from serologic surveillance data (Table 2). Yearling and older deer probably have higher rates than fawns because they have been bitten more times by infected ticks during their lifetimes. It has been demonstrated that yearling and adult deer have higher tick prevalence rates and higher tick burdens than fawns (12, 29). As the primary host for *I. scapularis*, deer, especially yearlings and adults, may experience hundreds, if not thousands, of bites per year from *I. scapularis*.

There was an excellent positive correlation between the ELISA and the Western immunoblotting results (Table 3). The average number of antigen bands detected in ELISA-positive samples from sites with established *I. scapularis* populations was 13.8; the corresponding value was only 3.0 for ELISA-negative samples from the same sites. For the five ELISA-positive samples tested from the sites lacking *I. scapularis*, the average number of bands was 8.2. These values for the ELISA-positive samples (13.8 and 8.2 bands) correlate well with their OD values (data not shown). Of the positive samples from sites without *I. scapularis*, two had OD values between 4 and 5 SDs above the baseline, two had OD values between 5 and 6 SDs above, and one had an OD value 8 and 9 SDs above. The OD values for the positive samples from areas with *I. scapularis* were considerably higher. Forty-four percent of these samples had OD values ≥6 SDs above the baseline, and 5% of them had OD values ≥12 SDs above. The presence of positive samples at the sites lacking *I. scapularis* may be explained as follows. (i) A few anti-*B. burgdorferi* antibody-positive deer from adjacent *I. scapularis*-infested areas moved into these collection sites. (ii) Serologic surveillance of deer is a more sensitive or efficient method of detecting the presence of *B. burgdorferi* in a geographic area than are methods involving tick collection by capturing small mammals or by drag cloth sampling. (iii) Dispersal by birds (27, 42) of *B. burgdorferi*-infected larval or nymphal *I. scapularis* into these sites occurred; in such a scenario, ticks would detach from the birds, molt into nymphs or adults, and then feed on local deer, an activity during which transmission of the spirochetes might occur.

Overall, a large number of different antigens (n = 58) were reactive in the Western immunoblot analysis of 365 samples. To establish with certainty the identities of reactive bands, five strips stained for protein with India ink and five strips probed with rabbit anti-*B. burgdorferi* serum were evenly distributed in each set of Western blot strips from every polyacrylamide gel. This technique is illustrated in Fig. 2, in which the results for Western blot strips from small sections of different polyacrylamide gels are shown, accompanied by their flanking India ink and rabbit control serum strips. With this method, the molecular masses of the antigens could be accurately identified for each set of strips tested.

We observed that 14 antigens were reactive with more than 50% of the ELISA-positive samples from sites with established populations of *I. scapularis* (Table 4). The recognition of many of these antigens has been reported for humans (2, 8, 11, 14, 16, 22, 32), rhesus monkeys (36), dogs (15), and hamsters (37). The antigens that are often reported in these other systems and that were observed in the present study to react with at least 50% of the ELISA-positive samples have molecular masses of 85, 55, 46, 43, 41 (flagellin), 39.5 (P39), 28.5 (OspD [34]), and 17 kDa. Additional antigens reactive with at least 50% of the positive samples in

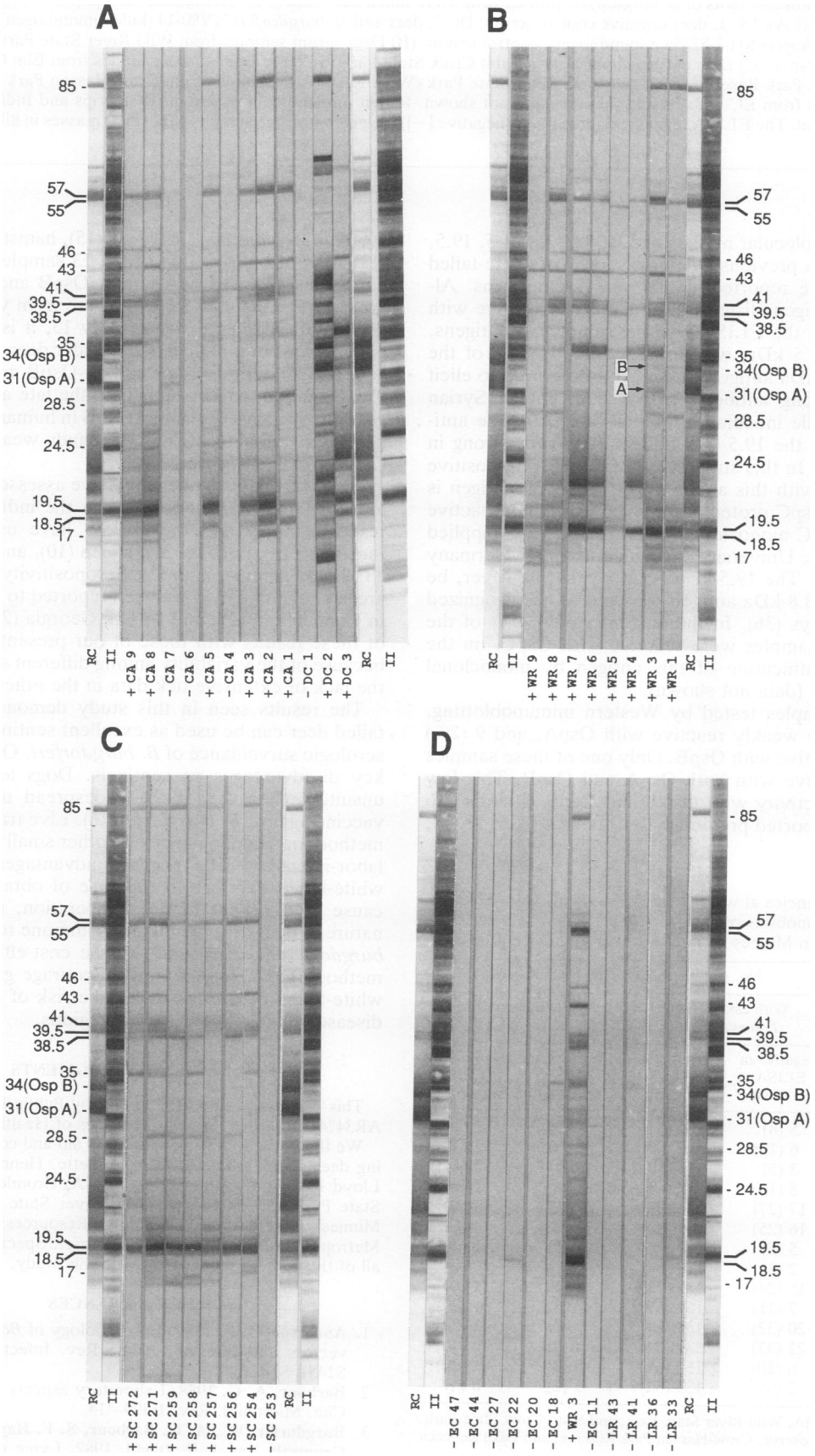
A
85
57
55
46
43
41
39.5
38.5
35
34(Osp B)
31(Osp A)
28.5
24.5
19.5
18.5
17
RC II + CA 9 + CA 8 − CA 7 − CA 6 + CA 5 − CA 4 + CA 3 + CA 2 + CA 1 − DC 1 + DC 2 + DC 3 RC II
B
B
A
RC II + WR 9 + WR 8 + WR 7 + WR 6 + WR 5 + WR 4 + WR 3 + WR 1 RC II
C
RC II + SC 272 + SC 271 + SC 259 + SC 258 + SC 257 + SC 256 + SC 254 − SC 253 RC II
D
RC II − EC 47 − EC 44 − EC 27 − EC 22 − EC 20 − EC 18 + WR 3 − EC 11 − LR 43 − LR 41 − LR 36 − LR 33 RC II

FIG. 2. Western immunoblots of *B. burgdorferi* probed with white-tailed deer sera or rabbit immune serum. (A) Deer serum samples from Carlos Avery WMA (CA); DC 1, deer negative control serum; DC 2, deer anti-*B. burgdorferi* NY90-14 (killed immunogen) control serum; DC 3, deer anti-*B. burgdorferi* SH-2-82 (live immunogen) control serum. (B) Deer serum samples from Wild River State Park (WR). The arrows identify OspB and OspA. (C) Deer serum samples from Saint Croix State Park (SC). (D) Deer serum samples from Elm Creek Park Reserve (EC), Lake Rebecca Park Reserve (LR), and Wild River State Park (WR). Results for samples from Crow-Hassan Park Reserve are similar to those for samples from EC and LR and therefore are not shown. Rabbit immune control serum (RC) strips and India ink (II) strips are included in each panel. The ELISA results (positive [+] or negative [−]) are given for each sample. Molecular masses in kilodaltons are shown on the left or right.

this study have molecular masses of 57, 38.5, 35, 24.5, 19.5, and 18.5 kDa. In a previous serologic survey of white-tailed deer sera (13), we reported similar reactive antigens. Although the 14 antigens listed in Table 4 were reactive with more than 50% of the ELISA-positive samples, 3 antigens, of 43, 41, and 19.5 kDa, were reactive with ≥80% of the samples. The 43-kDa antigen was recently reported to elicit an early and strong antibody response in golden Syrian hamsters by needle inoculation or tick bite (37). The antibody response to the 19.5-kDa antigen was very strong in white-tailed deer. In this study, 94% of the ELISA-positive samples reacted with this antigen. The 19.5-kDa antigen is not the 23-kDa OspC protein (30), since it was nonreactive with the anti-OspC monoclonal antibody L22 IF8, supplied by B. Wilske of the University of Munich, Munich, Germany (data not shown). The 19.5-kDa antigen may, however, be identical to the 18.8-kDa antigen reported to be recognized by rhesus monkeys (36). In the current study, 48% of the ELISA-positive samples were reactive with OspC, on the basis of the identification of this antigen by monoclonal antibody L22 IF8 (data not shown).

Of the 365 samples tested by Western immunoblotting, only 7 (2%) were weakly reactive with OspA, and 9 (2%) were weakly reactive with OspB. Only one of these samples (<1%) was reactive with both OspA and OspB. This low frequency of reactivity with OspA and OspB is similar to what has been reported previously for humans (2, 8, 11, 14, 16, 22, 32), monkeys (36), dogs (15), hamsters (37), and deer (13). It is of interest that of the 13 samples with detectable antibodies against OspA and/or OspB and for which there are deer age data, 11 samples were from yearling and adult deer. With a sample size of only 13, it is difficult to draw conclusions, but it appears that older deer are more likely to generate an anti-OspA or an anti-OspB antibody response. This situation may be similar to the late antibody response generated against OspA and OspB in humans (7). The arrows (A and B) in Fig. 2B indicate bands weakly reactive with OspA and OspB, respectively.

TABLE 4. Frequencies at which selected antigens were reactive in Western immunoblots probed with white-tailed deer serum samples collected in Minnesota in November and December 1992

Molecular mass (kDa) of antigen	No. (%) of reactive samples from sites			
	With established *I. scapularis*[a]		Without established *I. scapularis*[b]	
	Negative in ELISA (n = 63)	Positive in ELISA (n = 254)	Negative in ELISA (n = 43)	Positive in ELISA (n = 5)
85	5 (8)	169 (67)	1 (2)	1 (20)
57	6 (10)	139 (55)	0 (0)	2 (40)
55	3 (5)	146 (58)	2 (5)	2 (40)
46	8 (13)	157 (62)	1 (2)	2 (40)
43	17 (27)	223 (88)	3 (17)	3 (60)
41	16 (25)	204 (80)	11 (26)	4 (80)
39.5	5 (8)	179 (70)	4 (9)	2 (40)
38.5	2 (3)	138 (54)	2 (5)	0 (0)
35	15 (24)	182 (72)	8 (19)	4 (80)
28.5	7 (11)	169 (67)	0 (0)	1 (20)
24.5	20 (32)	138 (54)	1 (2)	1 (20)
19.5	21 (33)	239 (94)	4 (9)	2 (40)
18.5	6 (10)	155 (61)	0 (0)	1 (20)
17	2 (3)	133 (52)	1 (2)	0 (0)

[a] Carlos Avery WMA, Wild River State Park, and Saint Croix State Park.
[b] Elm Creek Park Reserve, Crow-Hassan Park Reserve, and Lake Rebecca Park Reserve.

Other serologic studies that have assessed the presence of anti-*B. burgdorferi* antibodies by the indirect immunofluorescence assay and the ELISA have used rodents (26), raccoons (25), dogs (9, 31), sheep (10), and deer (13, 23–25, 28, 31) as sentinel animals. Seropositivity rates for deer in areas of endemicity have been reported to be as high as 56% in Connecticut (25) and 36% in Georgia (28). A comparison of these results with those of our present study is difficult because of the variability among different serologic tests and the lack of extensive tick data in the other studies.

The results seen in this study demonstrate that white-tailed deer can be used as excellent sentinel animals for the serologic surveillance of *B. burgdorferi*. Other animals have key disadvantages as sentinels. Dogs are now generally unsuitable because of the widespread use of the canine vaccine against *B. burgdorferi* (6). Live trapping, a common method for sampling mice and other small mammals, is very labor-intensive. The primary advantages seen for using white-tailed deer are (i) the ease of obtaining samples because of excellent hunter cooperation, (ii) the sedentary nature of deer, (iii) the vigorous immune response against *B. burgdorferi* in deer, and (iv) the cost-effectiveness of this method. These reasons may encourage greater reliance on white-tailed deer in assessing the risk of contracting Lyme disease in specific geographic regions.

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REFERENCES

1. **Anderson, J. F.** 1989. Epizootiology of *Borrelia* in *Ixodes* tick vectors and reservoir hosts. Rev. Infect. Dis. **11**(Suppl. 6): S1451–S1459.
2. **Barbour, A. G.** 1988. Laboratory aspects of Lyme borreliosis. Clin. Microbiol. Rev. **1**:399–414.
3. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwalt, and J. P. David.** 1982. Lyme disease: a tick-borne

spirochetosis? Science **216:**1317–1319.
4. **Centers for Disease Control.** 1992. Distribution by county of *Ixodes dammini* in the United States, 1991. Lyme Dis. Surveill. Summ. **3:**1–5.
5. **Centers for Disease Control.** 1992. Notifiable diseases report. Morbid. Mortal. Weekly Rep. **40:**898–899.
6. **Chu, H.-J., L. G. Chavez, Jr., B. M. Blumer, R. W. Sebring, T. L. Wasmoen, and W. M. Acree.** 1992. Immunogenicity and efficacy study of a commercial *Borrelia burgdorferi* bacterin. J. Am. Vet. Med. Assoc. **201:**403–411.
7. **Coleman, J. L., and J. L. Benach.** 1987. Isolation of antigenic components from the Lyme disease spirochete: their role in early diagnosis. J. Infect. Dis. **155:**756–765.
8. **Craft, J. E., D. K. Fischer, G. T. Shimamoto, and A. C. Steere.** 1986. Antigens of *Borrelia burgdorferi* recognized during Lyme disease. J. Clin. Invest. **78:**934–939.
9. **Daniels, T. J., D. Fish, J. F. Levine, M. A. Greco, A. T. Eaton, P. J. Padgett, and D. A. LaPointe.** 1993. Canine exposure to *Borrelia burgdorferi* and prevalence of *Ixodes dammini* (Acari: Ixodidae) on deer as a measure of Lyme disease risk in the northeastern United States. J. Med. Entomol. **30:**171–178.
10. **Fridriksdottir, V., L. L. Nesse, and R. Gudding.** 1992. Seroepidemiological studies of *Borrelia burgdorferi* infection in sheep in Norway. J. Clin. Microbiol. **30:**1271–1277.
11. **Gill, J. S., and R. C. Johnson.** 1992. Immunologic methods for the diagnosis of infections by *Borrelia burgdorferi* (Lyme disease), p. 452–458. *In* N. R. Rose, E. C. de Macario, J. L. Fahey, H. Friedman, and G. M. Penn (ed.), Manual of clinical laboratory immunology, 4th ed. American Society for Microbiology, Washington, D.C.
12. **Gill, J. S., R. C. Johnson, M. K. Sinclair, and A. R. Weisbrod.** 1993. Prevalence of the Lyme disease spirochete, *Borrelia burgdorferi*, in deer ticks (*Ixodes dammini*) collected from white-tailed deer (*Odocoileus virginianus*) in Saint Croix State Park, Minnesota. J. Wildl. Dis. **29:**64–72.
13. **Gill, J. S., R. G. McLean, D. F. Neitzel, and R. C. Johnson.** 1993. Serologic analysis of white-tailed deer sera for antibodies to *Borrelia burgdorferi* by enzyme-linked immunosorbent assay and Western immunoblotting. J. Clin. Microbiol. **31:**318–322.
14. **Golightly, M. G., J. A. Thomas, and A. L. Viciana.** 1990. The laboratory diagnosis of Lyme borreliosis. Lab. Med. **21:**299–304.
15. **Greene, R. T., R. L. Walker, W. L. Nicholson, H. W. Heidner, J. F. Levine, E. C. Burgess, M. Wyand, E. B. Breitschwerdt, and H. A. Berkhoff.** 1988. Immunoblot analysis of immunoglobulin G response to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally exposed dogs. J. Clin. Microbiol. **26:**648–653.
16. **Grodzicki, R. L., and A. C. Steere.** 1988. Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. J. Infect. Dis. **157:**790–797.
17. **Hyde, F. W., and R. C. Johnson.** 1984. Genetic relationship of Lyme disease spirochetes to *Borrelia*, *Treponema*, and *Leptospira* spp. J. Clin. Microbiol. **20:**151–154.
18. **Johnson, R. (Minnesota Department of Natural Resources).** 1993. Personal communication.
19. **Johnson, R. C. (University of Minnesota).** 1993. Personal communication.
20. **Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Borrelia burgdorferi*: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. **34:**496–497.
21. **Jordan, P. A. (University of Minnesota).** 1993. Personal communication.
22. **Ma, B., B. Christen, D. Leung, and C. Vigo-Pelfrey.** 1992. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. J. Clin. Microbiol. **30:**370–376.
23. **Magnarelli, L. A., J. F. Anderson, W. Burgdorfer, and W. A. Chappell.** 1984. Parasitism by *Ixodes dammini* (Acari: Ixodidae) and antibodies to spirochetes in mammals at Lyme disease foci in Connecticut, USA. J. Med. Entomol. **21:**52–57.
24. **Magnarelli, L. A., J. F. Anderson, D. Fish, R. C. Johnson, and W. A. Chappell.** 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York state, and North Carolina. J. Wildl. Dis. **22:**178–188.
25. **Magnarelli, L. A., J. H. Oliver, Jr., H. J. Hutcheson, and J. F. Anderson.** 1991. Antibodies to *Borrelia burgdorferi* in deer and raccoons. J. Wildl. Dis. **27:**562–568.
26. **Magnarelli, L. A., J. H. Oliver, Jr., H. J. Hutcheson, J. L. Boone, and J. F. Anderson.** 1992. Antibodies to *Borrelia burgdorferi* in rodents in the eastern and southern United States. J. Clin. Microbiol. **30:**1449–1452.
27. **Magnarelli, L. A., K. C. Stafford III, and V. C. Bladen.** 1992. *Borrelia burgdorferi* in *Ixodes dammini* (Acari: Ixodidae) feeding on birds in Lyme, Connecticut, U.S.A. Can. J. Zool. **70:**2322–2325.
28. **Mahnke, G. L., D. E. Stallknecht, C. E. Greene, V. F. Nettles, and M. A. Marks.** 1993. Serologic survey for antibodies to *Borrelia burgdorferi* in white-tailed deer in Georgia. J. Wildl. Dis. **29:**230–236.
29. **Main, A. J., H. E. Sprance, K. O. Kloter, and S. E. Brown.** 1981. *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer (*Odocoileus virginianus*) in Connecticut. J. Med. Entomol. **18:**487–492.
30. **Marconi, R. T., D. S. Samuels, and C. F. Garon.** 1993. Transcriptional analyses and mapping of the *ospC* gene in Lyme disease spirochetes. J. Bacteriol. **175:**926–932.
31. **Mukolwe, S. W., A. A. Kocan, and J. H. Wyckoff III.** 1992. Serological survey for Lyme disease in domestic dogs and white-tailed deer from Oklahoma. Ann. N.Y. Acad. Sci. **653:** 172–177.
32. **Nadal, D., C. Taverna, and W. H. Hitzig.** 1989. Immunoblot analysis of antibody binding to polypeptides of *Borrelia burgdorferi* in children with different clinical manifestations of Lyme disease. Pediatr. Res. **26:**377–382.
33. **Neitzel, D. F., J. L. Jarnefeld, and R. D. Sjogren.** 1993. An *Ixodes scapularis* (deer tick) distribution study in the Minneapolis-St. Paul, Minnesota area. Bull. Soc. Vector Ecol. **18:**67–73.
34. **Norris, S. J., C. J. Carter, J. K. Howell, and A. G. Barbour.** 1992. Low-passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. Infect. Immun. **60:**4662–4672.
35. **Oliver, J. H., Jr., M. R. Owsley, H. J. Hutcheson, A. M. James, C. Chen, W. S. Irby, E. M. Dotson, and D. K. McLain.** 1993. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). J. Med. Entomol. **30:**54–63.
36. **Philipp, M. T., M. K. Aydintug, R. P. Bohm, Jr., F. B. Cogswell, V. A. Dennis, H. N. Lanners, R. C. Lowrie, Jr., E. D. Roberts, M. D. Conway, M. Karacorlu, G. A. Peyman, D. J. Gubler, B. J. B. Johnson, J. Piesman, and Y. Gu.** 1993. Early and early disseminated phases of Lyme disease in the rhesus monkey: a model for infection in humans. Infect. Immun. **61:**3047–3059.
37. **Roehrig, J. T., J. Piesman, A. R. Hunt, M. G. Keen, C. M. Happ, and B. J. B. Johnson.** 1992. The hamster immune response to tick-transmitted *Borrelia burgdorferi* differs from the response to needle-inoculated, cultured organisms. J. Immunol. **149:**3648–3653.
38. **Rongstad, O. J., and J. R. Tester.** 1969. Movements and habitat use of white-tailed deer in Minnesota. J. Wildl. Manage. **33:**366–379.
39. **Severinghaus, C. W.** 1949. Tooth development and wear as criteria of age in white-tailed deer. J. Wildl. Manage. **13:**195–216.
40. **Severinghaus, C. W., and E. L. Cheatum.** 1956. Life and times of the white-tailed deer, p. 57–186. *In* W. P. Taylor (ed.), The deer of North America. Stackpole Co., Harrisburg, Pa., and Wildlife Management Institute, Washington, D.C.
41. **Steere, A. C.** 1989. Lyme disease. N. Engl. J. Med. **321:**586–596.
42. **Weisbrod, A. R., and R. C. Johnson.** 1989. Lyme disease and migrating birds in the St. Croix River Valley. Appl. Environ. Microbiol. **55:**1921–1924.