Geographic Distribution of White-Tailed Deer with Ticks and Antibodies to Borrelia burgdorferi in Connecticut

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Ticks and blood specimens were collected from white-tailed deer (Odocoileus virginianus) in Connecticut and analyzed to identify foci for Lyme borreliosis. Males and females of Ixodes scapularis, the chief vector of Borrelia burgdorferi, were collected from deer in five of eight counties during 1989–1991. Analysis by indirect fluorescent antibody (IFA) staining of midgut tissues showed that prevalence of infection was highest (9.5% of 367 ticks) in south central and southeastern Connecticut. Infected I. scapularis also were collected from southwestern regions of the state (12.1% of 99 ticks), but prevalence of infection in northern counties was considerably lower (0.8% of 124 ticks). Deer sera, obtained in 1980 and 1989–1991, were analyzed by an enzyme-linked immunosorbent assay or by IFA staining methods. Antibodies to B. burgdorferi were detected in sera collected from all eight counties in Connecticut. Deer had been infected by this spirochete in at least 50 towns, 17 (34%) of which are in south central and southeastern parts of the state. Borrelia burgdorferi is widely distributed in I. scapularis populations in Connecticut.

Human cases of Lyme borreliosis have been reported from numerous towns in Connecticut [1–3]. Ixodes scapularis (previously designated Ixodes dammini[4]), the chief vector of Borrelia burgdorferi, is abundant in woodlands, particularly in south central and southeastern Connecticut [5–7]. In the past two decades, this tick's geographical range has expanded. Birds parasitized by larvae and nymphs have enhanced tick dispersal [8–10]. Other hosts, such as white-tailed deer (Odocoileus virginianus), white-footed mice (Peromyscus leucopus), Virginia opossums (Didelphis virginiana), and eastern chipmunks (Tamias striatus), are likewise parasitized by I. scapularis in forests [6,7,11–13]. Of these animals, white-footed mice are chief reservoirs for B. burgdorferi [12,14,15].

With continued reporting of human cases of B. burgdorferi infection and frequent media coverage of Lyme borreliosis, awareness of this disease has increased. Based on the occurrence of human cases, it is suspected that Lyme borreliosis has spread geographically. However, surveillance based solely on human case data can be misleading. It is often unclear where persons were bitten by infected ticks. Misdiagnosis also can occur. The characteristic expanding skin lesion, erythema migrans, does not always develop [16] and, in other instances, may not be recognized. Moreover, laboratory diagnosis can be inconclusive because of false positive or false

Abbreviations: ELISA: enzyme-linked immunosorbent assay  IFA: indirect fluorescent antibody

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negative serologic test results [17]. The objective of this study was to further identify foci for this disease in Connecticut by analyzing ticks and blood specimens collected from white-tailed deer. Deer are especially suitable for surveillance of Lyme borreliosis because they are important hosts for adults of I. scapularis [18], ticks and blood specimens can be easily obtained, and deer develop antibodies to B. burgdorferi [19–21].

MATERIALS AND METHODS

Ticks and blood specimens were collected from white-tailed deer killed during the fall hunting seasons of 1980 and 1989–1991. During examinations at official state deer checking stations, adults of I. scapularis were removed from the head areas of animals, and blood was collected from the body cavities. In 1980 an effort was made to examine deer from all eight counties in Connecticut, while during 1989–1991, emphasis was placed on the four northern counties. Information on sites where deer were killed in towns was provided by hunters to state personnel at the checking stations. Ticks were kept alive until they could be processed in the laboratory. Blood samples were centrifuged to obtain sera which were stored at −60 C until analysis.

Tick Analysis Midgut tissues were dissected from ticks and tested for B. burgdorferi by indirect fluorescent antibody (IFA) staining methods. Details on the use of murine monoclonal antibody (H5332) and fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G have been reported [22]. The monoclonal antibody was directed to outer surface protein A of B. burgdorferi, a polypeptide of about 31 kilodaltons [23,24] that is common to North American isolates of this bacterium. Ticks collected in 1980 could not be analyzed by these procedures because the monoclonal antibody was unavailable. Sampling during 1980 predated the discovery of B. burgdorferi [25].

SeroLogic Testing Serum specimens were analyzed for antibodies to B. burgdorferi by a newly developed enzyme-linked immunosorbent assay (ELISA) or by an IFA method [20,21]. Sera collected during 1980 were stored at −60 C and were available for analyses. Use of an ELISA facilitated seroanalyses and allowed for more efficient standardization of reagents. For these reasons most specimens tested during the entire study were analyzed by this method. In each test polyclonal conjugated antibodies were used. Therefore, antibody titers refer to total immunoglobulins. All analyses included positive and negative controls from previous work [19–21] and routine procedures to standardize antigens and newly purchased reagents. Additional positive and negative controls were provided by P. Luttrell of the Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Georgia. Sera were obtained from deer before and after inoculation of B. burgdorferi and were used in analyses to further check reactivity of antigen and conjugated reagents. Results on the sensitivity and specificity of our ELISA have been reported [20,21].

RESULTS

Adults of I. scapularis were collected from white-tailed deer in five counties during 1989–1991 (Table 1). Midgut tissues from 352 male ticks and 238 female ticks were tested for B. burgdorferi. Prevalence of infection was highly variable and ranged from 0% in Windham County to 26.1% for females collected in Middlesex County. In general, the numbers of I. scapularis collected and prevalences of infection were low in the northern counties of Connecticut.
Serologic test results confirmed deer exposure to *B. burgdorferi* at widely separated sites, including areas of northern Connecticut. In 1980, deer sera collected in the southern areas of Hartford, Tolland, and Windham Counties contained antibodies to *B. burgdorferi* (Fig. 1), but the number of seropositive deer was markedly greater in Middlesex and New London Counties. There was no evidence of deer exposure to *B. burgdorferi* in Litchfield County. Insufficient numbers of serum samples were col-

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**TABLE 1**

Number of Male and Female *Ixodes scapularis* Removed From White-Tailed Deer and Tested for *Borrelia burgdorferi* in Connecticut During 1989-1991

| Counties   | 1989 | 1990 | 1991 |
|------------|------|------|------|
|            | No. of Ticks Tested (%) Infected<sup>a</sup> | No. of Ticks Tested (%) Infected<sup>a</sup> | No. of Ticks Tested (%) Infected<sup>a</sup> |
|            | Males | Females | Males | Females | Males | Females |
| Fairfield  | NS<sup>b</sup> | NS<sup>b</sup> | 46 (10.9) | 25 (20) | 13 (7.7) | 15 (6.7) |
| Litchfield | NS   | NS   | 53 (0) | 11 (0) | 18 (0) | 15 (6.7) |
| Middlesex  | 21 (4.8) | 16 (25) | 54 (13) | 23 (26.1) | 132 (6.8) | 121 (6.6) |
| Tolland    | NS   | NS   | 6 (0) | 2 (0) | 3 (0) | 2 (0) |
| Windham    | 6 (0) | 8 (0) | NS | NS | NS | NS |

<sup>a</sup>Midgut tissues were removed from ticks and tested by indirect fluorescent antibody staining methods with murine monoclonal antibody (H5332).

<sup>b</sup>NS (Not Surveyed).

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**FIG. 1.** Distribution of deer with or without antibodies to *B. burgdorferi*, 1980.
lected in Fairfield and the northern section of New Haven Counties. Analyses of deer sera collected during 1989 and 1990 revealed past or current infections of *B. burgdorferi* in six of eight counties (Figs. 2 & 3). Antibodies to this spirochete were detected in Fairfield County, Litchfield County, the more northern areas of Tolland and Windham Counties, and in southeastern Connecticut. During the entire study, antibodies to *B. burgdorferi* were detected in sera collected from all eight counties in Connecticut (Table 2). Deer had been infected by *B. burgdorferi* in at least 50 towns, 17 (34%) of which are in Middlesex and New London Counties.

Prevalence of deer sera with antibodies to *B. burgdorferi* ranged from 13% in 1991 to 26% in 1980 and 1989 (Table 3) by an ELISA. Maximal antibody titers of 1:2560 were recorded during each year of sampling. In comparative analyses of sera collected during 1980, there was little difference in seropositivity as determined by an ELISA or the IFA staining method.

**DISCUSSION**

Based on tick collections and serologic test results, *I. scapularis* and *B. burgdorferi* are present at numerous locations in Connecticut, and deer are being exposed to this infectious agent statewide. These findings support surveillance records for human cases of Lyme borreliosis [2,3]. For example, the relatively higher numbers of infected ticks and seropositive deer in Middlesex County parallel incidence rates for human infections. The overall incidence of Lyme disease for Connecticut residents in 1988 was 22 per 100,000 [3]. The highest rates were among residents in south central and southeastern Connecticut (New London County: 108 per 100,000; Middlesex County: 22 per 100,000). Moreover, the greatest increase in incidence
between 1985 (2 per 100,000) and 1988 (14 per 100,000) occurred among residents of Fairfield County. Isolations of *B. burgdorferi* from white-footed mice there [26] coupled with serologic evidence of this spirochete in deer reaffirm that *B. burgdorferi* is present in numerous sites in southwestern Connecticut.

Prior to the discovery of *B. burgdorferi*, human cases of Lyme borreliosis were being

**TABLE 2**

Locations in Connecticut Where White-Tailed Deer Contained Antibodies to *Borrelia burgdorferi* in 1980 and 1989–1991 and Where Human Cases of Lyme Disease Have Been Reported

| Counties and Towns Where Deer Had Antibodies to *B. burgdorferi* |
|---------------------------------------------------------------|
| **New Fairfield** | **Haven** | **Middlesex** | **New London** | **Litchfield** | **Hartford** | **Tolland** | **Windham** |
| Bethel | Guilford | Chester | Colchester | Cornwall | Glastonbury | Ellington | Ashford |
| Newtown | Hamden | Durham | East Lyme | Kent | Marlborough | Hebron | Eastford |
| Redding | Madison | East Haddam | Griswold | Litchfield | Burlington | Tolland | Hampton |
| Ridgefield | East Hampton | Haddam | Lyme | New Hartford | Union | Plainfield |
| Weston | Killingworth | Old Lyme | N. Stonington | Montville | New Milford | Vernon | Woodstock |
| | Middletown | Salem | N. Canaan | Plymouth | | | |
| | Portland | | | | | | |

*Note: Based on epidemiological records in the Connecticut Department of Health Services, all towns listed except Union and Eastford have had reported human cases of Lyme borreliosis during 1989–1991.*
TABLE 3
Sera of White-Tailed Deer Tested for Antibodies to *Borrelia burgdorferi* in Connecticut during 1980 and 1989–1991

| Years | ELISA | IFA staining |
|-------|-------|--------------|
|       | Number Tested | No. (%) Positivea | Titers range | Number Tested | No. (%) Positivea | Titers range |
| 1980  | 66     | 17 (26) | 15%,36% | 160–2560 | 223 | 49 (22) | 17%,27% | 64–2048 |
| 1989  | 114    | 30 (26) | 18%,34% | 160–2560 | 0  | —   | —    | —     |
| 1990  | 193    | 29 (15) | 10%,20% | 160–2560 | 0  | —   | —    | —     |
| 1991  | 205    | 27 (13) | 9%,18%  | 160–2560 | 0  | —   | —    | —     |

aPositive antibody titers by an ELISA (≥ 1:160) or by IFA staining (≥ 1:64).
bCI (95% Confidence Intervals).
cResults published earlier [21] and listed here for comparison.

reported primarily from coastal areas or near the Connecticut River in south central and southeastern Connecticut [1]. Two subsequent articles indicate a more widespread geographic occurrence of human cases [2,3], including towns in the more northern sections of the state. Based on our analysis of deer sera from Litchfield County, it appears that *B. burgdorferi* infections became more prevalent there within the past decade. Elsewhere in Connecticut, numerous species of passerine birds have been found carrying infected larval and nymphal *I. scapularis* [7–10]. These hosts disperse *I. scapularis*. If deer, white-footed mice, and other forest-dwelling mammals are present in areas where infected, engorged ticks are introduced, new foci for Lyme borreliosis can be formed. Subsequently, amplification of *B. burgdorferi* can occur in sites if prevalence of infection increases in white-footed mouse populations. Serologic testing of deer sera is suitable for determining the presence or absence of Lyme borreliosis in forested areas, particularly if ticks removed from these hosts also can be analyzed for *B. burgdorferi*. Such testing is especially useful in areas where Lyme disease is newly established. Prevalence of seropositive deer, however, is variable and can be subject to sampling bias. In the present study, prevalence of deer with antibodies to *B. burgdorferi* declined from 26% in 1980 and 1989 to 13% and 15% during 1990 and 1991. Decreased seroprevalence was probably due to more extensive sampling during the latter two years in northern Connecticut where prevalence of Lyme disease is low. Ultimately, isolation of *B. burgdorferi* from these and other mammals and ticks is more desirable because successful culturing indicates direct evidence of infection and provides isolates that can be further studied for antigenic differences or pathogenicity. Although duration of antibody presence in deer is unknown, detection of these immunoglobulins in sera from these and other mammals indicates past exposure to *B. burgdorferi*. Seropositivity does not necessarily mean that these mammals are spirochetemic. White-tailed deer appear to be reservoir incompetent and, compared to white-footed mice, are believed to play little or no role in infecting ticks that feed on them [27]. Nonetheless, deer can be used to identify foci for *B. burgdorferi* infections because they are parasitized by infected immature and adult *I. scapularis* during different seasons, produce high concentrations of antibodies to *B. burgdorferi*, and, in some instances, live close to human residences.
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REFERENCES

1. Steere AC, Malawista SE: Cases of Lyme disease in the United States: Locations correlated with distribution of Ixodes dammini. Ann. Intern. Med. 91:730–733, 1979
2. Petersen LR, Sweeney AH, Checko PJ, Magnarelli LA, Mshar PA, Gunn RA, Hadler JL: Epidemiological and clinical features of 1,149 persons with Lyme disease identified by laboratory-based surveillance in Connecticut. Yale J Biol Med 62:253–262, 1989
3. Cartter ML, Mshar P, Hadler JL: The epidemiology of Lyme disease in Connecticut. Conn Med 53:320–323, 1989
4. Oliver JH, Jr., Owens MR, Hutcheson HJ, James AM, Chen C, Irby WS, Dotson EM, McLain DK: Conspecificity of the ticks Ixodes scapularis and I. dammini (Acari: Ixodidae). J Med Entomol 30:54–63, 1993
5. Anderson JF, Magnarelli LA: Vertebrate host relationships and distribution of ixodid ticks (Acari: Ixodidae) in Connecticut, USA. J Med Entomol 17:314–323, 1980
6. Carey AB, Kinsky WL, Main AJ: Ixodes dammini (Acari: Ixodidae) and associated ixodid ticks in south-central Connecticut, USA. J Med Entomol 17:89–99, 1980
7. Main AJ, Carey AB, Carey MG, Goodwin RH: Immature Ixodes dammini (Acari: Ixodidae) on small animals in Connecticut, USA. J Med Entomol 19:655–664, 1982
8. Anderson JF, Magnarelli LA: Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. Yale J Biol Med 57:627–641, 1984
9. Anderson JF, Johnson RC, Magnarelli LA, Hyde FW: Involvement of birds in the epidemiology of the Lyme disease agent Borrelia burgdorferi. Infect Immun 51:394–396, 1986
10. Anderson JF, Magnarelli LA, Stafford III KC: Bird-feeding ticks transstadially transmit Borrelia burgdorferi that infect Syrian hamsters. J Wildl Dis 26:1–10, 1990
11. Main AJ, Sprance HE, Kloter KO, Brown SE: Ixodes dammini (Acari: Ixodidae) on white-tailed deer (Odocoileus virginianus) in Connecticut. J Med Entomol 18:487–492, 1981
12. Anderson JF, Magnarelli LA, Burgdorfer W, Barbour AG: Spirochetes in Ixodes dammini and mammals from Connecticut. Am J Trop Med Hyg 32:818–824, 1983
13. Magnarelli LA, Anderson JF, Burgdorfer W, Chappell WA: 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, 1984
14. Donahue JG, Piesman J, Spielman A: Reservoir competence of white-footed mice for Lyme disease spirochetes. Am J Trop Med Hyg 36:92–96, 1987
15. Levine JF, Wilson ML, Spielman A: Mice as reservoirs of the Lyme disease spirochete. Am J Trop Med Hyg 34:355–360, 1985
16. Steere AC: Lyme disease. N Engl J Med 321:586–596, 1989
17. Magnarelli LA: Laboratory diagnosis of Lyme disease. Rheum Dis Clin North Am 15:735–745, 1989
18. Wilson ML, Adler GH, Spielman A: Correlation between abundance of deer and that of the deer tick, Ixodes dammini (Acari: Ixodidae). Ann Entomol Soc Am 78:172–176, 1985
19. Magnarelli LA, Anderson JF, Chappell WA: Antibodies to spirochetes in white-tailed deer and prevalence of infected ticks from foci of Lyme disease in Connecticut. J Wildl Dis 20:21–26, 1984
20. Magnarelli LA, Anderson JF, Apperson CS, Fish D, Johnson RC, Chappell WA: 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, 1986
21. Magnarelli LA, Oliver Jr JH, Hutcheson HJ, Anderson JF: Antibodies to Borrelia burgdorferi in deer and raccoons. J Wildl Dis 27:562–568, 1991
22. Magnarelli LA, Anderson JF: Ticks and biting insects infected with the etiologic agent of Lyme disease, Borrelia burgdorferi. J Clin Microbiol 26:1482–1486, 1988
23. Barbour AG, Tessier SL, Todd WJ: Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect Immun 41:795–804, 1983
24. Barbour AG, Heiland RA, Howe TR: Heterogeneity of major proteins in Lyme disease borreliae: A molecular analysis of North American and European isolates. J Infect Dis 152:478–484, 1985
25. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP: Lyme disease—a tick-borne spirochetosis? Science 216:1317–1319, 1982
26. Anderson JF, Mintz ED, Gadbaw JJ, Magnarelli LA: Babesia microti, human babesiosis, and Borrelia burgdorferi in Connecticut. J Clin Microbiol 29:2779–2783
27. Telford III SR, Mather TN, Moore SI, Wilson ML, Spielman A: Incompetence of deer as reservoirs of the Lyme disease spirochete. Am J Trop Med Hyg 39:105–109, 1988