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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 polyvalent 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.

TABLE 1
Number of Male and Female *Ixodes scapularis* Removed From White-Tailed Deer and Tested for *Borrelia burgdorferi* in Connecticut During 1989–1991

	1989		1990		1991	
	No. of Ticks Tested (%) Infected[a]		No. of Ticks Tested (%) Infected[a]		No. of Ticks Tested (%) Infected[a]	
Counties	Males	Females	Males	Females	Males	Females
Fairfield	NS[b]	NS[b]	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

[a]Midgut tissues were removed from ticks and tested by indirect fluorescent antibody staining methods with murine monoclonal antibody (H5332).
[b]NS (Not Surveyed).

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-

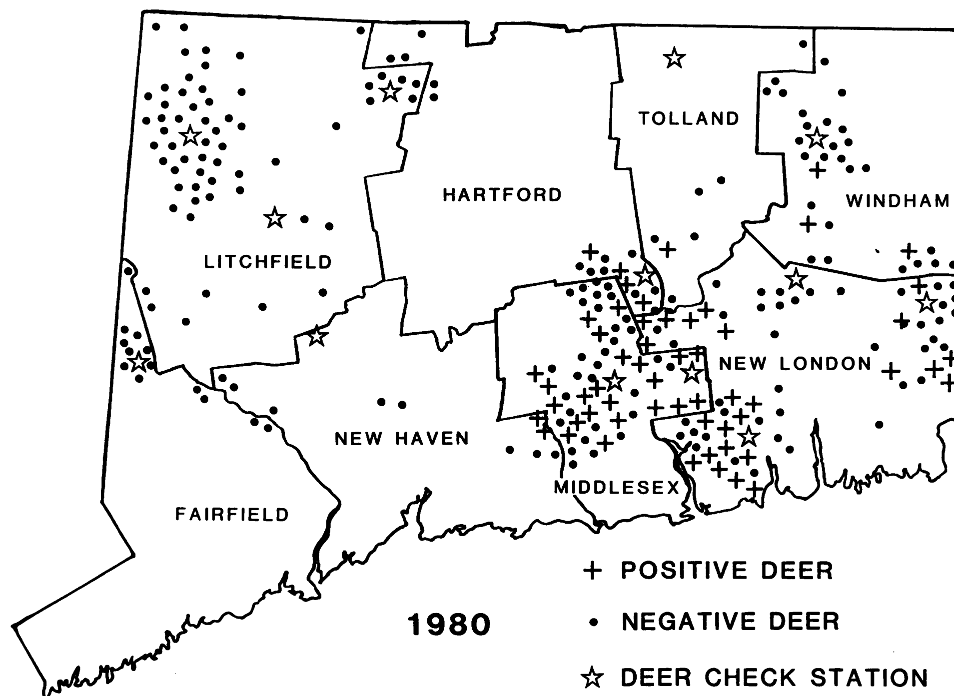

FIG. 1. Distribution of deer with or without antibodies to *B. burgdorferi*, 1980.

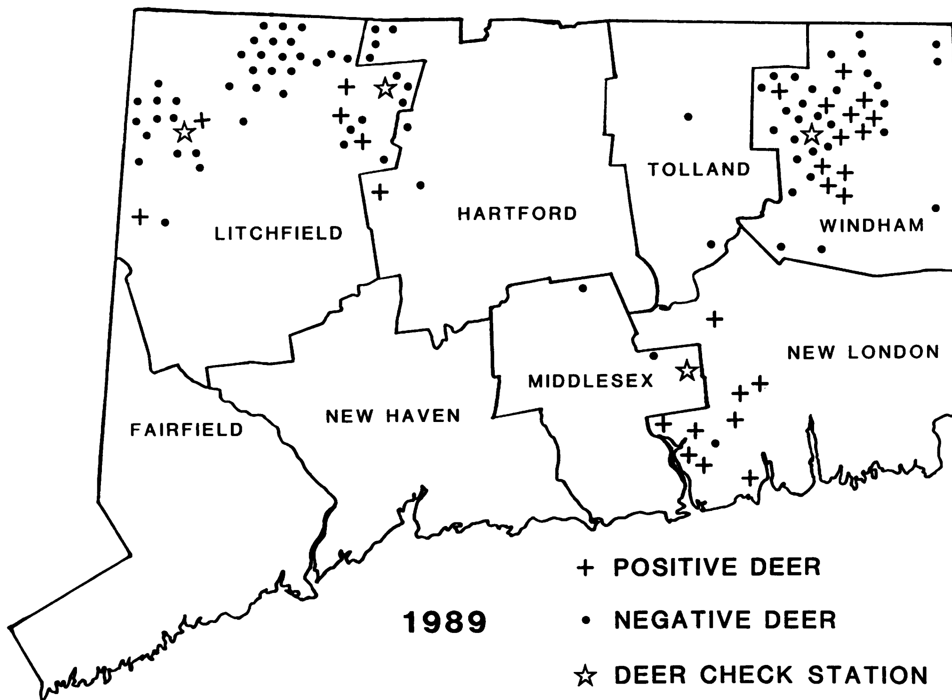

FIG. 2. Distribution of deer with or without antibodies to *B. burgdorferi*, 1989.

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

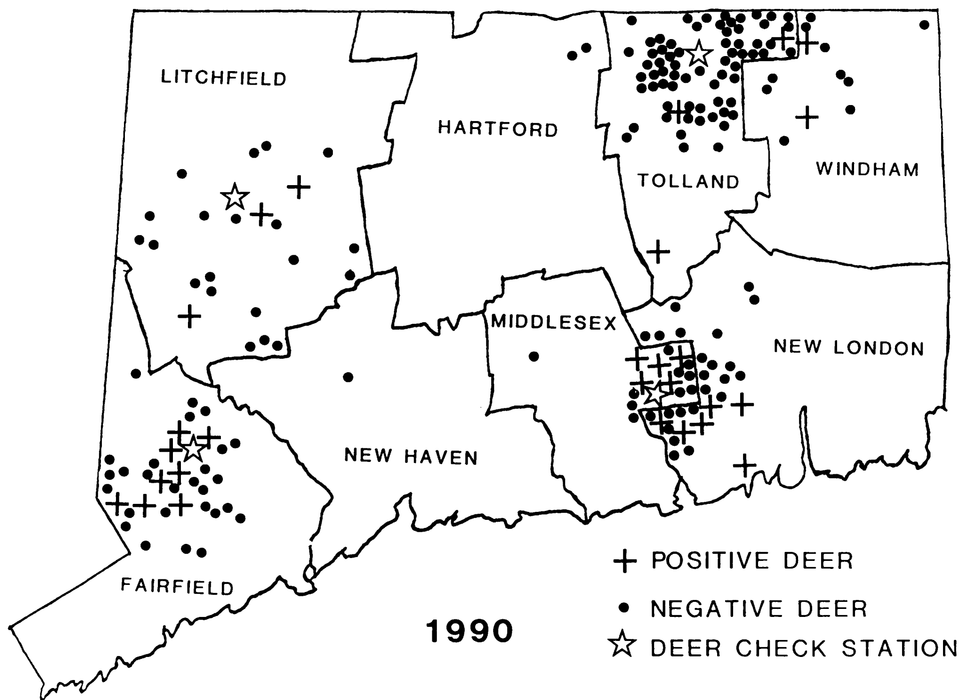

FIG. 3. Distribution of deer with or without antibodies to *B. burgdorferi*, 1990.

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*							
Fairfield	New 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	Lyme	New Hartford		Union	Plainfield
Weston		Haddam	Montville	New Milford		Vernon	Pomfret
		Killingworth	N. Stonington	N. Canaan		Willington	Scotland
		Middletown	Old Lyme	Plymouth			Woodstock
		Portland	Salem	Sharon			
			Voluntown	Winchester			

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

	ELISA				IFA staining			
Years	Number Tested	No. (%) Positive[a]	CI[b]	*Titers* range	Number Tested	No. (%) Positive[a]	CI[b]	*Titers* range
1980	66	17 (26)	15%,36%	160–2560	223	49 (22)	17%,27%	64–2048
1989[c]	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	—		—

[a]Positive antibody titers by an ELISA (≥ 1:160) or by IFA staining (≥ 1:64).
[b]CI (95% Confidence Intervals).
[c]Results 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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