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Borrelia burgdorferi in an Urban Environment: White-Tailed Deer with Infected Ticks and Antibodies

LOUIS A. MAGNARELLI,[1]* ANTHONY DENICOLA,[2] KIRBY C. STAFFORD III,[1] AND JOHN F. ANDERSON[1]

Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504,[1] and Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 49707[2]

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Ticks and blood samples were collected from white-tailed deer (*Odocoileus virginianus*) in forests located in an insular, urban area of Bridgeport, Conn., and in rural south central Connecticut during 1992 and 1993. Immature and adult *Ixodes scapularis* ticks were tested for *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis, by indirect fluorescent-antibody staining methods. Deer sera were analyzed for antibodies to this bacterium by an enzyme-linked immunosorbent assay. Infected ticks parasitized deer in Bridgeport from May through December; the prevalence of infection varied from 1.1% of 93 larvae to 28.1% of 114 adult females. The percentages of infected males (10.5% of 380 ticks) and females (13.7% of 328 ticks) were relatively lower in south central Connecticut. In antibody tests, the prevalence of seropositive specimens collected in Bridgeport (61% of 146 serum specimens) was more than twofold greater than that of specimens obtained in south central Connecticut (26.7% of 116 serum specimens). Foci for Lyme borreliosis can occur in forested, urban settings as well as in rural areas if there are ticks, rodents, birds, and large mammals present. Human exposure to ticks in such sites should be considered as a possible source of *B. burgdorferi* infection.

White-tailed deer (*Odocoileus virginianus*) create several problems when they attain high population densities. The correlation between rising populations of deer and the tick *Ixodes scapularis* in the northeastern United States is well documented (2, 6, 12, 16, 17, 27). This tick transmits *Borrelia burgdorferi* sensu stricto, the etiologic agent of Lyme borreliosis, and parasitizes several species of mammals and birds (6). *B. burgdorferi* is maintained in rodents, particularly *Peromyscus leucopus* (white-footed mouse), while deer appear to be preferred hosts for adult *I. scapularis* ticks (28). Therefore, problems with Lyme borreliosis may occur in areas subjected to urban and suburban development as well as in isolated park and forest preserves where deer, rodents, and birds can thrive.

The occurrence of this multisystem disease in rural and suburban environments is well recognized by public health officials. Numerous reports describe the ecology and epidemiology of Lyme borreliosis in these settings (1–3, 5, 10, 11, 15, 17, 18, 22, 24). However, little is known about the occurrence of *B. burgdorferi* in ticks and mammals or the risk of acquiring Lyme borreliosis in forested, urban environments. Tick vectors and pathogens may be prevalent if host densities are high and other ecological factors favor tick survival. This report describes a focus for this disease in an insular, forested site in Bridgeport, the largest city in Connecticut.

MATERIALS AND METHODS

Study sites. A privately owned forest located in Bridgeport, Conn., was chosen for collection of ticks and blood samples from white-tailed deer during 1992 and 1993. This site consists of about 176 ha of predominantly mixed deciduous trees surrounded by extensive tracts of human residences. A 2.5-m-high wire fence around the perimeter of the forest prevents the deer from leaving the private property. This woodland study area is essentially an island in an urban setting. During 1992, there were about 128 deer there. The deer population was reduced to 68 individuals in 1993 as a part of ongoing research and management projects at the site. In addition to numerous species of passerine birds, common mammals, such as white-footed mice, raccoons (*Procyon lotor*), woodchucks (*Marmota monax*), and gray squirrels (*Sciurus carolinensis*), inhabit the woodlands.

For comparison, ticks and blood samples were collected from white-tailed deer killed during the hunting seasons in south central and southeastern Connecticut in November and December of 1992 and 1993. The deer were examined at state check stations located in East Haddam and Haddam. Unlike the Bridgeport site, the East Haddam and Haddam forests are far more extensive, and deer densities are lower because of lack of confinement. The exact deer densities in the East Haddam and Haddam sites are unknown.

Collecting ticks and deer blood. At the Bridgeport site, some deer were temporarily immobilized with tiletamine hydrochloride and zolazepam hydrochloride (Telazol; Elkins-Sinn, Inc., Cherry Hill, N.J.) via a tranquilizer gun at a dose of 3.3 mg/kg of body weight. Xylazine hydrochloride (Mobay Corp., Shawnee, Kans.) was administered subsequently at 2.2 mg/kg of body weight. Other deer were killed. Ticks were removed primarily from the heads of the animals and were placed into vials containing blades of grass for moisture. Blood samples were collected from the cephalic veins of tranquilized deer or from the hearts of killed deer and were kept cool until centrifugation in the laboratory. The tranquilized deer were tagged for identification, monitored until full recovery, and allowed to return unharmed to their natural habitat. In the laboratory, ticks were kept at 20°C until analyses for *B. burgdorferi* could be performed. Sera were stored at −60°C until serologic testing for antibodies could be conducted.

At deer check stations, adult ticks were collected from the heads of deer, while blood samples were obtained from body cavity areas. Blood samples were taken from some animals that had been dead for 6 to 8 h. Specimens were kept cool while in transit to the laboratory and were processed in the same manner as those collected in Bridgeport.

Testing ticks. Immature and adult ticks were analyzed for *B. burgdorferi* by indirect fluorescent-antibody staining methods. Midgut and other tissues were dissected from live unengorged or partially engorged ticks and smeared onto glass microscope slides. Procedures for acetone fixation, for application of murine monoclonal antibody (H5332) directed to outer surface protein A of *B. burgdorferi*, and for the use of a 1:60 dilution of fluorescein-conjugated polyvalent goat anti-mouse immunoglobulin G (heavy- and light-chain-specific) antibodies (Organon Teknika Corp., Durham, N.C.) as the second antibody have been reported previously (18). Preparations were examined for spirochetes by fluorescence microscopy.

Serologic analyses. Deer sera were analyzed for total immunoglobulins to *B. burgdorferi* by an enzyme-linked immunosorbent assay (ELISA). Details regarding the materials and procedures used in these analyses and results of sensitivity and specificity tests have been published elsewhere (22). In brief, serum dilutions of 1:160, 1:320, and 1:640 were screened in initial tests. If positive at 1:640, sera were reanalyzed to determine titration endpoints. A commercially developed reagent, horseradish peroxidase-labeled rabbit anti-deer immunoglobulins (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.), was used in all

* Corresponding author. Mailing address: Department of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504-1106. Phone: (203) 789-7241. Fax: (203) 789-7232.

TABLE 1. Numbers of white-tailed deer examined for *I. scapularis* and prevalence of ticks infected with *B. burgdorferi* in Bridgeport, Conn.

Yr	Sampling period	Total no. of deer examined[a]	No. (%) of deer parasitized	*I. scapularis* removed from deer and infected with *B. burgdorferi*[b]							
				Larvae		Nymphs		Males		Females	
				No. tested	% Positive	No. tested	% Positive	No. tested	% Positive	No. tested	% Positive
1992	February–April	88	0	0		0		0		0	
	May–July	19	11 (57.9)	2	0	8	0	14	14.2	15	0
	August–September	34	30 (88.2)	93	1.1	31	6.5	32	21.9	70	7.1
	October–December	15	15 (100)	0		2	0	101	24.8	114	28.1
1993	June–July	5	0	0		0		0		0	
	August–September	28	19 (67.9)	1	0	0		0		1	0
	October–December	14	14 (100)	0		0		45	4.4	111	7.2

[a] Totals include 36 deer recaptured in 1992.
[b] Tick midgut tissues were tested by indirect fluorescent-antibody staining with monoclonal antibody H5332.

analyses. Enzyme-labeled antibodies and other reagents were tested with the positive and negative controls to determine optimal working dilutions and to ensure standardization.

RESULTS

Immature and adult *I. scapularis* ticks parasitized deer in Bridgeport from May through December. Of the 203 deer examined in 1992 and 1993, 89 (43.8%) had ticks attached (Table 1). The prevalence of infected ticks varied from 1.1% of 93 larvae to 28.1% of 114 adult females. The former was recorded during peak larval abundance during late August and early September. Adult ticks also were recovered from deer in late August and September but were more abundant from October through December. Male and female ticks also parasitized deer in early May, while nymphs were found on deer from summer into early August.

Adult *I. scapularis* ticks were collected from deer in south central Connecticut in November and December of 1992 and 1993. Of the 316 deer examined, 46.5% were found to be carrying ticks (Table 2). The prevalence of infected males (10.5%) and females (13.7%) in 1992, however, was lower than what was recorded in Bridgeport (males, 24.8%; females, 28.1%) during the same sampling period. In 1993, the prevalences of infected ticks in the two sites were comparable (range, 2.8 to 8.1%).

White-tailed deer in Bridgeport and south central Connecticut had antibodies to *B. burgdorferi*. The prevalence of seropositive specimens in Bridgeport (61.0% of 146 serum specimens) was more than twofold greater than that recorded in seroanalyses of specimens collected in south central Connecticut (26.7% of 116 serum specimens) during 1992 (Table 3). Antibodies were detected in serum samples from deer in Bridgeport during each month of sampling. Seropositivity was highest (73.8%) for samples obtained from February through April and was lowest (22.2%) from May through July. Antibody titers ranged from 1:160 to 1:2,560, except for sera collected in May through July (range, 1:320 to 1:640). On the basis of geometric mean titers, sera had relatively high concentrations of antibodies to *B. burgdorferi* from February through April ($\bar{x}$ = 226) and had markedly lower concentrations of antibodies from May through July ($\bar{x}$ = 69).

Blood samples were obtained from 31 deer (23 males and 8 females) recaptured from February through November of 1992 in Bridgeport. Recapture periods ranged between 1 and 8 months. Seroconversions (i.e., changes from negative to positive status) were noted for three males and one female, while reversions (i.e, positive to negative changes) were recorded for paired serum samples from four males and one female. There were no changes in serologic reactivity of the remaining 22 pairs of serum specimens tested from 16 male and 6 female deer. Fourteen pairs of serum samples (10 male, 4 female) were positive in both tests, while the remaining eight pairs of serum samples (6 male, 2 female) were negative in analyses.

In 1993, 43 serum samples were collected from deer in Bridgeport from June through November; 7 samples (16.3%) were positive, with titration endpoints ranging between 1:160 and 1:640. In south central Connecticut, 65 (35.5%) of 183 serum samples, collected during November and December, had antibodies to *B. burgdorferi*. Antibody titers ranged between 1:160 and 1:1,280.

TABLE 2. Numbers of white-tailed deer examined for *I. scapularis* and prevalence of ticks infected with *B. burgdorferi* in south central Connecticut

Yr	Sampling period	Total no. of deer examined	No. (%) of deer parasitized	*I. scapularis* removed from deer and infected with *B. burgdorferi*[a]			
				Males		Females	
				No. tested	No. (%) positive	No. tested	No. (%) positive
1992	November	62	52 (83.9)	248	27 (10.9)	213	35 (16.4)
	December	65	36 (55.4)	132	13 (9.9)	115	10 (8.7)
	Total	127	88 (69.3)	380	40 (10.5)	328	45 (13.7)
1993	November	140	47 (33.6)	161	14 (8.7)	55	0 (0)
	December	49	12 (24.5)	36	2 (5.6)	17	2 (11.8)
	Total	189	59 (31.2)	197	16 (8.1)	72	2 (2.8)

[a] See Table 1, footnote *b*.

TABLE 3. Reactivity of white-tailed deer sera in an ELISA for antibodies to *B. burgdorferi*[a]

Sampling period[a]	Bridgeport, Conn., specimens				South central Connecticut specimens			
	No. tested[b]	No. (%) positive	Antibody titer		No. tested	No. (%) positive	Antibody titer	
			Geometric x̄	Range			Geometric x̄	Range
February–April	84	62 (73.8)	226	160–2,560	0			
May–July	18	4 (22.2)	69	320–640	0			
August–September	31	17 (54.8)	187	160–2,560	0			
October–December	13	6 (46.2)	136	160–2,560	116	31 (26.7)	71	160–640
Total	146	89 (61.0)	186	160–2,560	116	31 (26.7)	71	160–640

[a] Serum samples were collected in 1992.
[b] Total includes 31 serum samples collected from recaptured deer.

DISCUSSION

Foci for Lyme borreliosis can occur in urban settings if *I. scapularis* and deer, white-footed mice, and other key forest-dwelling animals are present. Deer are the chief hosts for adult *I. scapularis* ticks (27, 28), while white-footed mice are the main reservoirs for *B. burgdorferi* (2, 12, 16). It is unclear when *I. scapularis* and *B. burgdorferi* became established at the Bridgeport site. Deer have been present at this location for at least 70 years. In Fairfield, Conn., a town adjacent to Bridgeport, *B. burgdorferi* was isolated from white-footed mice in 1985 (4). With fencing in place for at least 50 years at the Bridgeport site, we suspect that passerine birds may have played a role in introducing infected ticks to that area. Many species of birds have been found carrying larval and nymphal *I. scapularis* (3, 8, 9, 23, 26); *B. burgdorferi* has been isolated from a veery (*Catharus fuscescens*) in Connecticut (3). Moreover, clusters of infected *I. scapularis* larvae have been removed from birds, such as veeries, Carolina wrens (*Thryothorus ludovicianus*), hooded warblers (*Wilsonia citrina*), and house wrens (*Troglodytes aedon*) (23). Some birds, therefore, may serve to infect ticks as well as to transport them to new sites. Alternatively, *I. scapularis* and *B. burgdorferi* may have been present at the Bridgeport site for several decades, and other animals, such as white-footed mice and raccoons, also may have introduced ticks and *B. burgdorferi* to this area.

The prevalence of infected ticks parasitizing deer varies geographically (14, 20, 24). However, the prevalence of *B. burgdorferi* infection in adult *I. scapularis* ticks collected from deer in south central Connecticut in 1992 is relatively consistent and comparable to results obtained earlier. During November and December of 1982 through 1984, 11.4% of 281 male and 10.3% of 233 female ticks removed from deer in the Haddam and East Haddam, Conn., check stations were infected (19, 21). Similarly, 8.2% of 207 males and 11.2% of 160 females taken from deer in Middlesex County, which includes Haddam and East Haddam, during the same months of 1989 through 1991 contained *B. burgdorferi* (20). Surprisingly, percentages of infected adult *I. scapularis* ticks in the confined and limited forested ecological setting in Bridgeport were greater than those for ticks collected in the rural, more expansive woodland areas in southeastern and south central Connecticut. The incidence of human Lyme borreliosis infections in these rural, forested communities has been reported to be 108 cases per 100,000 population (10). When conditions are suitable, the prevalence of infected *I. scapularis* can be relatively high in localized suburban and rural settings (25) and in urban environments. Consequently, there is potential for human Lyme borreliosis infections in diverse settings.

The proportion of deer sera with antibodies to *B. burgdorferi* in south central Connecticut also has been relatively consistent over a 10-year period. The prevalence of seropositive specimens in the present study (26.7% of 116 serum specimens) was comparable to those recorded for deer sera collected at the same check stations from 1982 through 1983 (28.3% of 360 serum samples [17, 19]) and in 1990 and 1991 (26.2% of 103 serum samples [17a, 20]). The reason for the relatively higher prevalence of seropositive deer in Bridgeport and elsewhere is unclear, but results probably indicate more-frequent exposure of deer to infected ticks. To a lesser extent, higher seropositivity values for Bridgeport specimens also might be due to the collection of better-quality serum specimens. The recovery of sizable numbers of adult *I. scapularis* ticks from deer in late August and September in Bridgeport was surprising. Host-seeking adult *I. scapularis* ticks are normally more abundant on vegetation in south central Connecticut in October or later months (25).

At other insular, urban sites where deer are not abundant, other hosts, such as cottontail rabbits (*Sylvilagus floridanus*), and ticks (*Ixodes dentatus*) infected with *B. burgdorferi* have been found. These spirochetes were cultured from subadult *I. dentatus* ticks removed from lagomorphs captured in another urban environment, at the New York Botanical Garden in New York, N.Y. (7). Although *I. dentatus* does not readily feed on human beings, it apparently can maintain *B. burgdorferi* in foci. If other tick vectors, such as *I. scapularis*, become established in these areas, larval or nymphal ticks might acquire this bacterium from rabbits and subsequently transmit the agent to other hosts. The ecological aspects of Lyme borreliosis are complex and probably include closely related strains of *B. burgdorferi* sensu stricto in different enzootic cycles.

Residents of cities and their surrounding areas contract Lyme borreliosis. However, it is often assumed that these persons were exposed to infected ticks in rural or suburban settings, in which the occurrence of this spirochetosis is well documented. Established foci for *B. burgdorferi* exist in the greater Philadelphia, Pa., area (1). Tick bites also occur in recreational parks (13). When travel histories are unclear relative to the onset of illness, as they usually are, then epidemiological evidence of Lyme borreliosis can be misleading. In unique urban environments such as Bridgeport, Conn., where deer, rodents, birds, and ticks abound in forested parks or similar settings, human exposure to tick-borne agents, such as *B. burgdorferi*, is possible. Urban foci for Lyme borreliosis should be the subject of further study.

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