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SPIROCHETES IN TICKS AND ANTIBODIES TO *BORRELIA BURGDORFERI* IN WHITE-TAILED DEER FROM CONNECTICUT, NEW YORK STATE, AND NORTH CAROLINA

Louis A. Magnarelli,[1] John F. Anderson,[1] Charles S. Apperson,[2] Durland Fish,[3] Russell C. Johnson,[4] and W. Adrian Chappell[5]

ABSTRACT: Ticks were screened for spirochetes and serum samples from white-tailed deer (*Odocoileus virginianus*) were assayed for antibodies to *Borrelia burgdorferi* during 1983–1984. Using fluorescein isothiocyanate-labeled rabbit antibodies produced to *B. burgdorferi*, the etiologic agent of Lyme disease, spirochetes were detected in *Ixodes dammini* (10.5% of 1,193) and *Dermacentor albipictus* (0.6% of 157) adults from Connecticut, *I. dammini* nymphs (49.1% of 108) and adults (64.7% of 99) from Armonk, New York, and in *I. scapularis* (0.4% of 531) and *Amblyomma americanum* (3.5% of 173) adults from North Carolina. Infected ticks were either seeking hosts or feeding on deer during the summer and fall. Direct fluorescent antibody staining also revealed spirochetes in two larvae of *I. scapularis* that emerged from eggs deposited by separate females in the laboratory. Using indirect immunofluorescence tests, antibodies to *B. burgdorferi* were identified in white-tailed deer living in tick-infested areas of all three states. Aside from minor cross-reactivity, there was no serologic evidence of *Treponema* or *Leptospira* infections. *Ixodes dammini* is a primary vector of *B. burgdorferi* in northeastern United States, but in North Carolina, other ixodid ticks may transmit this spirochete to humans and wildlife.

INTRODUCTION

The etiologic agent of Lyme disease, *Borrelia burgdorferi* (Johnson et al., 1984a, b) has been isolated and identified from the ticks *Ixodes dammini* (Burgdorfer et al., 1982; Anderson et al., 1983; Steere et al., 1983b; Johnson et al., 1984), *I. pacificus* (Burgdorfer et al., 1985), *I. ricinus* (Barbour et al., 1983b), and *Dermacentor variabilis* (Anderson et al., 1985). It has also been cultured from the tissues of humans (Benach et al., 1983; Steere et al., 1983b) and wild mammals (Anderson et al., 1983, 1985; Bosler et al., 1983; Loken et al., 1985). Morphologic and immunochemical analyses of several isolates, including the type strain (B31) from *I. dammini*, verified that they belong to the same species (Hyde and Johnson, 1984; Johnson et al., 1984a, b; Schmid et al., 1984). Similar spirochetes have been detected in *Amblyomma americanum* (Schulze et al., 1984), insects (Anderson and Magnarelli, 1984), and a dog (Lissman et al., 1984). The extensive exposure of wildlife and domestic animals to this bacterium is further demonstrated by the presence of serum antibodies (Magnarelli et al., 1984a, b).

Although studies have revealed spirochetes in immature and adult ticks collected at foci of Lyme disease, little is known about temporal differences in infection prevalences for ticks or about changes in the number of seropositive mammals at widely separated sites. Therefore, this investigation was conducted to determine if the prevalence of infected ticks varies with the species, life stage, geographic setting, or with sampling in different seasons and to determine if the proportion of serum samples with antibodies to *B. burgdorferi* varies at foci.

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[1] Department of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504, USA.

[2] Department of Entomology, North Carolina State University, P.O. Box 7613, Raleigh, North Carolina 27695, USA.

[3] Department of Biological Sciences, Fordham University, Bronx, New York 10458, USA.

[4] Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455, USA.

[5] Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333, USA.

MATERIALS AND METHODS

Sampling procedures

Ticks and serum samples were collected during 1983 and 1984 from rural areas in Connecticut, New York State, and North Carolina. The following sites, all within the geographic range of *I. dammini*, were chosen for study in Connecticut: Haddam, East Haddam, East Lyme, Marlborough, Voluntown, and West Stafford. Human cases of Lyme disease have been reported from the first four communities (Steere and Malawista, 1979; Magnarelli et al., 1984c). For comparison, Litchfield and Sharon were included during 1983 to represent areas in northwestern Connecticut, where *I. dammini* has not been found. Each site had an official state check station during the fall hunting seasons. Personnel of the State Wildlife Unit removed ticks from freshly killed deer in November and December and collected serum samples as described earlier (Magnarelli et al., 1984a, b). Questing (i.e., host-seeking) larvae and nymphs of *I. dammini* were collected from dry-ice baited traps (Wilson et al., 1972) in foci, where human cases of Lyme disease were reported, during June through September at Armonk, New York (Westchester County). In addition, adult ticks were removed from a dog as they crawled on the fur or were obtained by "flagging" vegetation near woodlands during June, July, October, and December. In North Carolina, adults of *I. scapularis* and all motile stages of the lone star tick, *A. americanum*, were collected from white-tailed deer during August, September, and December in the counties of Beaufort, Jones, Onslow, Robeson, and Wake. Ticks and serum samples were shipped to The Connecticut Agricultural Experiment Station (C.A.E.S.) for analyses.

Six fully engorged females of *I. scapularis*, obtained in Onslow County on 12 December 1983, were placed into glass tubes containing moist filter paper and cotton plugs. These specimens were maintained at 12 ± 3 C until they deposited eggs and larvae emerged. Samples of larval ticks were smeared on to glass microscope slides and processed for direct fluorescent antibody (FA) staining.

Examination of ticks for spirochetes

To determine prevalence of infection, midgut tissues were removed from live ticks and were smeared on glass microscope slides. Following acetone treatment for 10 min, preparations were overlaid with high-titered fluorescein isothiocyanate (FITC)-labeled rabbit antibody (diluted 1:100) produced against *B. burgdorferi*. At this dilution, the conjugated antibody stained *Borrelia* organisms intensely, reacted weakly to treponemes, and was non-reactive against a series of serovars of *Leptospira interrogans*. Direct FA staining was used also to demonstrate spirochetes in the anterior section of the digestive tract (region of the pharyngeal cavity and esophagus), midgut, and in hindgut tissues of representative nymphs and adults. Dissections were performed under a stereomicroscope with watchmaker's forceps and a series of probing needles (i.e., various sizes of insect pins) mounted in wooden applicator sticks. To expose the pharyngeal cavity and esophagus, an incision was made by applying sharpened needles to the ventral portion of the basis capitulum. Tissues in the opened area, which extended posteriorly from the base of the hypopharynx, were smeared on glass microscope slides. Hindgut tissues were obtained by removing sections of the cuticle anterior to the anus, while portions of the midgut were excised dorsally as described earlier (Anderson et al., 1983). All slides were incubated with conjugated antibody for 30 min at 37 C, washed in phosphate buffered saline (PBS) solutions, mounted in buffered glycerol, and examined with a Zeiss fluorescence microscope.

Serologic analyses for antibodies

Serum samples were assayed for antibodies to *B. burgdorferi* by indirect immunofluorescence (IF) procedures modified from the methods of Philip et al. (1976). Details on the preparation of whole cell antigens, serum titrations, and on the source and dilution of FITC-labeled rabbit antisera to total white-tailed deer immunoglobulins (Ig) have been reported (Burgdorfer et al., 1982; Anderson et al., 1983; Magnarelli et al., 1984a, b). Antibody titers of ≥1:64 were considered positive, with endpoints referring to the highest titers for which there was definite fluorescence of spirochetes. Assays included antigen, conjugate, and negative and positive serum controls. To standardize the procedures, the same high and low-titered control serum samples were used in each test.

In serologic analyses, a local strain of *B. burgdorferi* (CT #2591) was used. This spirochete, originally cultured from the blood of a white-footed mouse (*Peromyscus leucopus*) captured at East Haddam, Connecticut, was morphologically and serologically indistinct from a series of other isolates of *B. burgdorferi* (including the B31 type strain, ATCC 35210) recovered from mammals and *I. dammini* (Anderson et al., 1983; Magnarelli et al., 1984a, b). In addition, murine monoclonal antibodies (H5332),

TABLE 1. Total numbers of *Ixodes dammini* removed from white-tailed deer and screened by direct fluorescent antibody tests for *Borrelia* spirochetes in Connecticut during 1983 and 1984.

Check station	No. adults tested						No. (%) FA positive					
	1983			1984			1983			1984		
	Totals	♂	♀	Totals	♂	♀	Totals	♂	♀	Totals	♂	♀
Haddam	55	31	24	124	57	67	9 (16.4)	2 (6.5)	7 (29.2)	5 (4.0)	1 (1.8)	4 (6.0)
East Haddam	166	110	56	147	61	86	28 (16.9)	23 (20.9)	5 (8.9)	9 (6.1)	4 (6.6)	5 (5.8)
East Lyme	82	51	31	117	58	59	19 (23.2)	15 (29.4)	4 (12.9)	16 (13.7)	10 (17.2)	6 (10.2)
Marlborough	180	122	58	186	90	96	21 (11.7)	10 (8.2)	11 (19.0)	5 (2.7)	1 (1.1)	4 (4.2)
Voluntown	0	—	—	131	52	79	0	—	—	13 (9.9)	1 (1.9)	12 (15.2)
West Stafford	2	1	1	3	0	3	0	—	—	0	—	—

Note: Representative tick specimens have been deposited in the United States National Museum under entry numbers RML116867–116869.

directed against the 31,000 molecular weight surface protein of *B. burgdorferi* (Barbour et al., 1983c, 1984), reacted positively in indirect IF tests using fluorescein-labeled mouse IgG antibodies.

Serum samples from deer were screened in IF tests against *B. burgdorferi*, a relapsing fever spirochete (*B. hermsii*-strain HS1 serotype C), two treponemes found in humans (*Treponema denticola* and *T. pallidum*), and the following serovars of *Leptospira interrogans*: *ballum* (strain Mus 127), *canicola* (strain Moulton), *grippotyphosa* (strain SC 4397), *hardjo* (strain Hardjoprajitno), *icterohemorrhagiae* (strain CF-1), and *pomona* (strain MLS). Stock antigens of *Leptospira* and *T. denticola* were prepared by fixing cultured material in 0.25% formalin for 2 hr, centrifuging at 12,000 *g* for 10 min, and by resuspending the supernatant in saline. Lyophilized *T. pallidum* and the corresponding rabbit antiserum, supplied by the Centers for Disease Control in Atlanta, Georgia 30333, USA, were resuspended in distilled water before testing. *Borrelia hermsii* antigen and rabbit antiserum were provided by A. G. Barbour of the Rocky Mountain Laboratories (Hamilton, Montana 59840, USA), while the remaining antigens and antisera were produced at the University of Minnesota. Aliquots of suspended stock antigens were mixed 1:1 with 3% yolk sac and were tested against reference rabbit antisera in homologous and heterologous reactions. Fluorescein-labeled and unconjugated goat anti-rabbit total Ig were obtained commercially (Grand Island Biological Company, Grand Island, New York 14072, USA) and were diluted in PBS (pH = 7.2) ≥1:50.

RESULTS

Spirochete infections in ticks

During 1983 and 1984, 1,193 adults of *I. dammini* were collected from 343 white-tailed deer in Connecticut. Of these, 125 (10.5%) harbored spirochetes that reacted with FITC-labeled rabbit antisera to *B. burgdorferi*. Prevalence of infection for males and females varied during both years (Table 1), but values for each sex were lower in 1984 than in 1983.

A total of 157 nymphs and adults of *Dermacentor albipictus* were removed from 66 white-tailed deer at Haddam, E. Haddam, Marlborough, and W. Stafford. A single female, collected from Haddam

TABLE 2. Number of questing immature and adult *Ixodes dammini* collected in Armonk, New York and screened by direct fluorescent antibody tests for *Borrelia* spirochetes.

	No. larvae		No. nymphs		No. males		No. females	
Sampling dates	Tested by FA	Positive	Tested by FA	Positive	Tested by FA	Positive	Tested by FA	Positive
16 Dec. 1983	0		0		32	23 (72%)	18	10 (56%)
7 June 1984	60	0	50	26 (52%)	5	2 (40%)	5	4 (80%)
12 July 1984	0		46	21 (46%)	0		1	0
16 Aug. 1984	0		12	6 (50%)	0		0	
12–20 Sept. 1984	25	0	0		0		0	
19 Oct. 1984	0		0		34	24 (71%)	4	1 (25%)
Totals	85	0	108	53 (49.1%)	71	49 (69%)	28	15 (53.6%)

during 1983, contained spirochetes in its midgut. Voucher specimens of this species were deposited in the United States National Museum (Smithsonian Institution, Washington, D.C. 20560, USA) under entry numbers RML 116863 and 116864.

Questing adult and immature *I. dammini*, obtained in Armonk, New York, also contained spirochetes. Of the 99 adults tested, 64.7% were positive (Table 2). Infected males and females were collected in June, October, and December of 1983 or 1984. Nearly 50% of the 108 nymphs examined also harbored spirochetes. These ticks were prevalent during June, July, and August. However, none of the 85 larvae, captured in dry-ice baited traps in June and September, were infected.

Dissections were performed to determine if spirochetes lived throughout the digestive tracts of *I. dammini*. Of the 56 nymphs and adults collected in Armonk and found to have spirochetes in their midguts, 36 (64.3%) also had the organisms in the region of the pharyngeal cavity and esophagus. Three females (5.4%) harbored spirochetes in the anterior of digestive tracts, the midgut, and in the posterior hindgut regions (i.e., anal area). Similar examinations of 17 positive adults, collected from white-tailed deer in southeastern Connecticut, revealed spirochetes in areas of the pharyngeal cavity, esophagus, and in the midguts of six females and one male. Three additional females had these bacteria throughout their digestive tracts.

Spirochetes were detected in two adults of *I. scapularis* and seven specimens of *A. americanum* removed from 149 white-tailed deer in Onslow County, North Carolina. Prevalences of infection (0.4% and 3.5%) were recorded for 531 adults of the former and 173 of the latter, respectively (Table 3); members of each sex were infected. A single nymph of *A. americanum*, collected on 31 August 1984, also had spirochetes.

Each of the six fully engorged females of *I. scapularis* from Onslow County, North Carolina deposited eggs in the laboratory, and within 4 wk, larvae emerged. Two of 92 (2.2%) larvae contained spirochetes. Prevalence of infection ranged from one of 25 and one of seven larvae representing the offspring of two females. Spirochetes were not detected in 60 larvae (n = 15 from each tick) that emerged from the eggs deposited by the remaining four females. Adult females decomposed soon after laying eggs and were not examined for spirochetes.

Antibodies in white-tailed deer

Antibodies to *B. burgdorferi* were detected in 141 (16.7%) of the 846 serum samples obtained from deer in three states. Of the 631 samples tested from Connect-

TABLE 3. Number of specimens of *Ixodes scapularis* and *Amblyomma americanum* collected from white-tailed deer in North Carolina and screened for *Borrelia* spirochetes by direct fluorescent antibody tests.

Site (county)	Sampling dates	No. deer examined	*I. scapularis* ♂ Total tested	*I. scapularis* ♂ No. pos.	*I. scapularis* ♀ Total tested	*I. scapularis* ♀ No. pos.	*A. americanum* Larvae Total tested	*A. americanum* Larvae No. pos.	*A. americanum* Nymphs Total tested	*A. americanum* Nymphs No. pos.	*A. americanum* ♂ Total tested	*A. americanum* ♂ No. pos.	*A. americanum* ♀ Total tested	*A. americanum* ♀ No. pos.
Onslow	12 Dec. 1983	42	69	0	36	0	—		—		—		—	
	31 Aug. 1984	6	—[a]		—		88	0	62	1	—		—	
	15–22 Dec. 1984	101	267	1	159	1	0		4	0	110	4	11	2
Beaufort	13–14 Aug. 1984	6	—		—		72	0	68	0	17	0	3	0
Jones	13–14 Aug. 1984	6	—		—		6	0	36	0	32	0	—	
Wake	16–27 Sept. 1984	7	—		—		3	0	—		—		—	
Robeson	23 Aug. 1984	1	—		—		—		—		—		—	
	18–29 Sept. 1984	4	—		—		—		—		—		—	
	Totals	173	336	1	195	1	169		170	1	159	4	14	2

[a] No ticks collected.

Note: Representative specimens of *I. scapularis* (RML 116865, 116866, 116944, and 116945) and *A. americanum* (RML 117466–117468) have been deposited in the United States National Museum.

icut, 112 (17.8%) had titers ranging from 1:64 to 1:16,384 (Table 4). Although prevalences for Connecticut, Armonk, New York (83%, n = six samples tested), and North Carolina (11.5%, n = 209) varied, titration endpoints usually ranged between 1:64 and 1:512. Only five serum samples, all collected in Connecticut, reacted at dilutions ≥1:1,024. Positive serum samples were obtained from six sites in Connecticut and from five counties in North Carolina: Beaufort (n = one of six samples), Jones (one of six), Onslow (19 of 185), Robeson (one of five), and Wake (two of seven). Seropositivity in Haddam, E. Haddam, E. Lyme, Marlborough, and Voluntown, areas where *I. dammini* is abundant, ranged between 13.8% and 39.3%. Serum samples obtained in West Stafford, an area in northcentral Connecticut where only five *I. dammini* were collected from 132 deer, also contained antibodies to *B. burgdorferi*. However, none of the 30 serum samples collected at Litchfield and Sharon in northwestern Connecticut was positive.

Tests were conducted to determine the specificity of our IF procedures. Of the 63

TABLE 4. Number of serum samples from white-tailed deer with antibodies to *Borrelia* spirochetes in Connecticut during 1983 and 1984.

Check station	Total sera tested 1983	Total sera tested 1984	No. (%) positive 1983	No. (%) positive 1984	No. samples at reciprocal titers 64–128	256–512	1,024–2,048	≥4,096
Litchfield[a]	5	—[b]	0	—				
Sharon[a]	25	—	0	—				
Haddam	29	28	4 (13.8)	11 (39.3)	10	5		
East Haddam	75	79	14 (18.7)	21 (26.6)	21	10	4	
East Lyme	37	51	7 (18.9)	10 (19.6)	14	3		
Marlborough	44	64	8 (18.2)	15 (23.4)	12	10		1
Voluntown	—	62	—	14 (22.6)	12	2		
West Stafford	62	70	2 (3.2)	6 (8.6)	5	3		

[a] Sampling areas not considered foci of Lyme disease.

[b] — No serum samples collected.

TABLE 5. Reactivity of positive serum samples from white-tailed deer to *Borrelia burgdorferi*, *B. hermsii*, *Treponema denticola*, and *Leptospira interogans* antigens.

No. positive samples to *Borrelia burgdorferi*[a]	Reciprocal antibody titers to *B. burgdorferi*	No. positive samples and antibody titer				
				Leptospira interrogans serovars		
		Borrelia hermsii	*Treponema denticola*	*pomona*	*hardjo*	*icterohemorrhagiae*
20	64	8 (64)	1 (64)	1 (64)		
15	128	9 (64–128)	3 (64)		1 (64)[c]	
14	256	6 (64–256)	2 (64)[b]	1 (64)[c]		1 (64)[c]
8	512	7 (64–512)	5 (64)[b]			
5	1,024	6 (64–1,024)	2 (64)[b]		1 (128)[c]	1 (128)[c]
1	16,384	1 (16,384)	1 (128)[b]			

[a] Positive = ≥1:64; samples collected from Haddam (n = 13), E. Haddam (n = 16), E. Lyme (n = 8), Marlborough (n = 11), W. Stafford (n = 3), Voluntown (n = 1), and Onslow County, North Carolina (n = 11).

[b] Also reacted with *T. pallidum* at 1:64–1:128.

[c] Also reacted with *L. interrogans* serovars *ballum*, *canicola*, and *grippotyphosa* at 1:64–1:128.

serum samples from white-tailed deer with antibodies to *B. burgdorferi*, 37 (58.7%) reacted with *B. hermsii* (Table 5). Fourteen samples had equivalent titration endpoints to both antigens. There was also cross-reactivity with *T. denticola* (n = 14 samples) and with one or more of the serovars of *Leptospira* (n = six samples). However, titration endpoints for 14 of these were at least four-fold lower than those to *B. burgdorferi*. In no instance did antibody titers to *B. hermsii*, the treponemes, or leptospires exceed those of *B. burgdorferi*. An additional 106 serum samples, collected at seven sites in Connecticut (n = 76) and in Onslow County, North Carolina (n = 30) and found to be nonreactive to *Borrelia* antigens, were screened against treponemes and leptospires; results were negative. Analyses of the reference rabbit antisera revealed homologous titration endpoints of ≥1:16,384 (Table 6). Heterologous titers for both *Borrelia* antigens were two to four-fold lower than the homologous titers. Endpoints for the remaining heterologous reactions were at least four-fold lower than the homologous titers.

DISCUSSION

Borrelia spirochetes were found in members of all four species of ticks. Results for Connecticut and New York State support previous studies (Burgdorfer et al., 1982; Anderson et al., 1983; Magnarelli et al., 1984b), which established *I. dammini* as a primary vector of *B. burgdorferi*. The low prevalence of infected specimens of *D. albipictus* suggests that this tick, like *D. variabilis* (Anderson and Magnarelli, 1984; Anderson et al., 1985), probably ingested spirochetes from infected hosts but has a minor role in the ecology of Lyme disease. Likewise, the low prevalence of infection (0.4%) noted for *I. scapularis*, the juvenile stages of which will feed on reptiles or mammals (Rogers, 1953), is comparable to those reported by Burgdorfer (1984) for *I. pacificus* in Oregon (2% positive) and in northcentral California (0.9% positive). Our finding infected specimens of *A. americanum* in North Carolina is consistent with observations made in New Jersey (Schulze et al., 1984) and reaffirms that ixodid ticks, other than those of the *I. ricinus* complex, may also harbor *B. burgdorferi*.

In addition to white-tailed deer, immatures and adults of *I. dammini* will feed on a wide range of mammals and birds. In northeastern United States, spirochetes have been isolated from or detected in humans, a dog, white-footed mice, a meadow vole (*Microtus pennsylvanicus*), a wood-

TABLE 6. Reactivity of reference rabbit antisera to antigens of *Borrelia*, *Treponema*, and *Leptospira interrogans* in indirect IF tests.

	Rabbit antisera									
					Serovars of *Leptospira interrogans*					
Spirochetes	*B.b.*[a]	*B.h.*	*T.d.*	*T.p.*	*ballum*	*canicola*	*grippo-typhosa*	*hardjo*	*ictero-haemor-rhagiae*	*pomona*
Borrelia burgdorferi[a] (2591)	32,768[b]	4,096	128	256	0	0	0	64	64	0
Borrelia hermsii	8,192	16,384	128	256	0	0	0	0	0	0
Treponema denticola	4,096	128	16,384	512	0	0	0	64	0	0
Treponema pallidum	4,096	256	128	16,384	0	64	0	64	64	0
Leptospira interrogans serovars										
ballum	0[c]	0	0	0	8,192	256	256	256	128	128
canicola	0	0	0	0	128	16,384	256	512	1,024	512
grippotyphosa	0	0	0	0	128	256	16,384	128	128	128
hardjo	0	0	0	0	64	256	256	16,384	256	128
icterohaemorrhagiae	0	0	0	0	256	1,024	128	256	16,384	128
pomona	0	0	0	0	128	256	512	1,024	256	16,384

[a] Rabbit antiserum was produced at Yale University against the original Shelter Island (B31) isolate from *I. dammini* (Steere et al., 1983b). Antigen (2591) was isolated from a white-footed mouse in Connecticut. *B.b.* = *Borrelia burgdorferi*, *B.h.* = *Borrelia hermsii*, *T.d.* = *Treponema denticola*, *T.p.* = *Treponema pallidum*.

[b] Expressed as reciprocal antibody titers. The staggered step line delineates homologous titers.

[c] 0 = titers < 64; based on assays of negative controls (using goat anti-rabbit Ig) with test antigens.

land jumping mouse (*Napaeozapus insignis*), a raccoon (*Procyon lotor*), white-tailed deer, eastern chipmunks (*Tamias striatus*), and six species of passeriforms (Anderson et al., 1983; Benach et al., 1983; Bosler et al., 1983, 1984; Steere et al., 1983b; Anderson and Magnarelli, 1984; Lissman et al., 1984; Anderson et al., 1985; Levine et al., 1985). Application of murine monoclonal antibodies, produced against surface proteins of the type strain of *B. burgdorferi*, and DNA homology studies of other available strains isolated in Connecticut (Barbour et al., 1983c; Hyde and Johnson, 1984; Johnson et al., 1984a, b; Schmid et al., 1984; Anderson et al., 1985) have verified that these spirochetes were *B. burgdorferi*. Rodents and birds, important hosts for larvae and nymphs of *I. dammini* (Anderson and Magnarelli, 1984), may be serving as reservoirs for this agent in the northeastern United States.

Adults of *I. dammini*, collected in southcentral and southeastern Connecticut, contained spirochetes, but prevalence of infection in 1984 was consistently lower than in 1983. Although differences were also noted in the percentages of serum samples with antibodies to *B. burgdorferi*, results obtained in 1984 for E. Haddam, E. Lyme, and Haddam paralleled more closely the prevalences recorded for these sites in 1982 (Magnarelli et al., 1984b). Therefore, the numbers of infected ticks and serum samples with antibodies can vary greatly within and among foci.

The collection of positive specimens of *I. dammini* from West Stafford in northern Connecticut is a new state record marking an inland distribution. Members of 27 species of birds have been found thus far carrying larvae and nymphs of *I. dammini* (Anderson and Magnarelli, 1984). Compared to mammals, these vertebrates are probably more instrumental in disseminating immatures of *I. dammini* over broad geographical areas and in establishing new foci for Lyme disease infections. Based on early and modern distribution records, Spielman et al. (1979, 1984, 1985) have concluded that the range of this tick is expanding.

Spirochetes were detected in regions of the pharyngeal cavity and esophagus, midguts, and hindguts of immatures and adults of *I. dammini*. These findings are similar to those of Burgdorfer et al. (1982) and indicate that of these areas, the midgut is most suitable for spirochete detection. However, infections with *Borrelia* may be generalized in ticks (Burgdorfer, 1984), and other tissues or organs, such as central nerve ganglia and ovaries, may also contain these agents. Although transovarial transmission of spirochetes occurs in *I. scapularis*, the efficiency of this process is undetermined. Nonetheless, the presence of spirochetes in anterior and posterior portions of the digestive tract means that transmission may also occur by feeding or by posterior station (i.e., spirochetes enter skin tissues of the host after being excreted from the tick). The precise mode of transmission to vertebrate hosts is unknown.

Serologic studies should be conducted in conjunction with the isolation and identification of spirochetes to locate Lyme disease foci and to monitor spirochetal activity in wildlife populations. Strong cross-reactivity with *B. hermsii*, the causative agent of tick-borne relapsing fever in western United States (Burgdorfer, 1976), can occur. Therefore, the coexistence of *B. burgdorferi* and *B. hermsii* in areas where these or other closely related *Borrelia* are present may confuse the interpretation of serologic results. In addition, antigenic cross-reactivity has been reported between members of *Borrelia* and *Treponema* (Barbour et al., 1983a; Baker-Zander and Lukehart, 1984). Our positive deer sera reacted weakly with treponemes and leptospires when antibody titers to *B. burgdorferi* exceeded 1:256. Aside from

this, there was no evidence of *Leptospira* infections, and as previously reported (Hyde and Johnson, 1984; Schmid et al., 1984), we conclude that there is minor antigenic relatedness between these spirochetes and *B. burgdorferi* and that the antibodies detected in deer were specific to *B. burgdorferi*.

Human infections of Lyme disease have been acquired in several areas of Connecticut and New York State where *I. dammini* abounds (Steere and Malawista, 1979; Benach et al., 1983; Steere et al., 1983a, b; Hanrahan et al., 1984; Magnarelli et al., 1984c, d). A small number of cases have been reported also from North Carolina (Pegram et al., 1983; Harshbarger et al., 1984) and from western states (Steere and Malawista, 1979; Burgdorfer and Keirans, 1983). Since the low number of Lyme disease cases in North Carolina, California, and Oregon correlates well with prevalences of infection for *I. scapularis* and *I. pacificus*, the epizootiology and epidemiology of Lyme disease may be correspondingly different in these areas. Therefore, further studies are needed to determine vector competence of these three closely related species of *Ixodes*.

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