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ANTIBODIES TO SPIROCHETES IN WHITE-TAILED DEER AND PREVALENCE OF INFECTED TICKS FROM FOCI OF LYME DISEASE IN CONNECTICUT

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ABSTRACT: White-tailed deer (*Odocoileus virginianus*) were examined for the tick, *Ixodes dammini*, and sera were analyzed for antibodies to spirochetes during 1982. Of the 323 animals inspected in four areas endemic for Lyme disease, 188 (58%) had adult ticks; parasitism ranged from 43% at Haddam to 82% at East Lyme. Direct and indirect fluorescent antibody tests detected spirochetes in 18 of 133 (14%) ticks. Indirect immunofluorescence tests revealed antibodies at titers of 1:64–1:4,096 to this bacterium in 93 (28%) of the 332 sera assayed. There is a close correlation among the distribution of spirochete-infected *I. dammini*, deer with antibodies, and human cases of Lyme disease.

INTRODUCTION

Lyme disease is a newly recognized human illness found in widely separated regions of the United States (Steere and Malawista, 1979). The etiologic agent is thought to be a spirochete (Burgdorfer et al., 1982) transmitted by closely related ticks of the *Ixodes ricinus* complex (Steere and Malawista, 1979). Members of this group include *Ixodes dammini*, a newly described species (Spielman et al., 1979), *Ixodes pacificus*, and *I. ricinus*. Expanding annular skin lesions, erythema chronicum migrans (ECM), may develop within 20 days after tick bites and, weeks or months later, may be followed by other disorders such as migratory polyarthritis, recurrent attacks of oligoarticular arthritis, chronic arthritis, or neurologic or cardiac abnormalities (Steere et al., 1978; Steere and Malawista, 1979). Immunoglobulins in acute and convalescent sera from persons with one or more of these manifestations bound in indirect immunofluorescence (IF) tests with spirochetes isolated from *Ixodes dammini*, a raccoon (*Procyon lotor*), and a white-footed mouse (*Peromyscus leucopus*) (Burgdorfer et al., 1982; Anderson et al., 1983; Steere et al., 1983).

Serologic analyses of raccoons, white-footed mice, dogs, white-tailed deer, eastern chipmunks (*Tamias striatus*), Virginia opossums (*Didelphis virginiana*), and gray squirrels (*Sciurus carolinensis*) revealed antibodies to spirochetes that may cause Lyme disease (Magnarelli et al., 1984). Although seropositivity prevalences varied among species (10–50%), there was a close correlation between the distributions of *I. dammini* and mammals with antibodies. Since white-tailed deer are important hosts for this tick (Piesman et al., 1979; Anderson and Magnarelli, 1980; Main et al., 1981) and harbor these bacteria (Bosler et al., 1983), they may play an important role in the ecology of Lyme disease. We report here parasitism by *I. dammini*, prevalence of spirochete-infected ticks, and antibodies to spirochetes in white-tailed deer.

MATERIALS AND METHODS

The following sites were chosen for sampling during 1982: East Haddam, East Lyme, Haddam, Voluntown, Litchfield and Sharon. Each area has woodlands and forest-grassland transitional zones containing deer. The first four sites are in southcentral or eastern Connecticut (Fig. 1) where *I. dammini* is abundant and where

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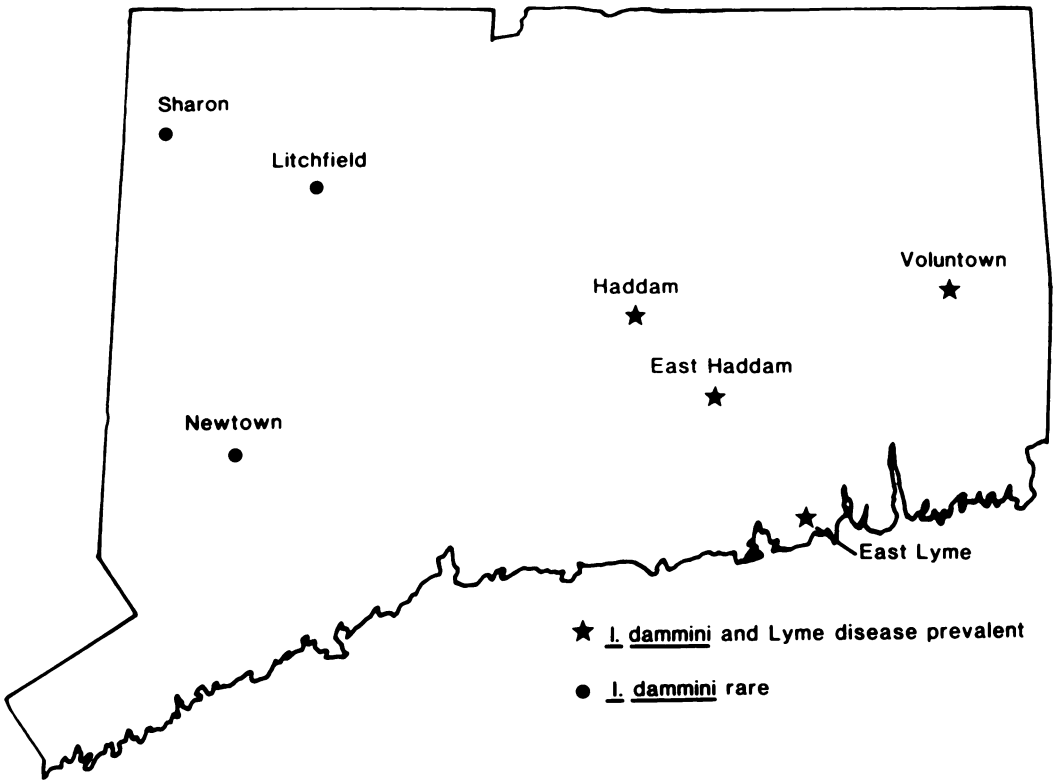

FIGURE 1. Sites in Connecticut where *Ixodes dammini* and Lyme disease are prevalent or rare.

Lyme disease is endemic (Wallis et al., 1978; Steere and Malawista, 1979). Litchfield and Sharon are in northwestern Connecticut, a region where this tick is rare and where human cases of Lyme disease have not been reported.

Freshly killed deer were brought to official state check stations during the hunting season (November and December). During a 10-min inspection period, ticks were removed principally from the head areas and were placed into sealed plastic vials. Blood samples were drawn from body cavities and centrifuged to obtain sera.

Live ticks were screened for spirochetes in hemolymph tests (Burgdorfer, 1970) by using a direct application of high-titered fluorescein isothiocyanate (FITC)-labeled rabbit antibody prepared to these organisms. Fluorescent-antibody (FA) positive and negative ticks were then dissected as described by Anderson et al. (1983) to determine if the midguts were infected with spirochetes, a technique demonstrated by Burgdorfer et al. (1982). Tissues were smeared on glass microscope slides, fixed in acetone for 10 min, and tested by direct FA or indirect IF procedures (i.e., overlaid with high-titered human sera followed by incubation, washings in phosphate buffer solutions (pH = 7.2), and treatments with FITC-conjugated goat anti-human immunoglobulins (Ig)). Conjugates for direct FA were prepared at Yale University and were diluted 1:100 in phosphate buffer. FITC-labeled reagents used in indirect IF tests with human sera were obtained commercially (Baltimore Biological Laboratories, Cockeysville, Maryland 21030, USA) and were diluted $\geq$1:80. Slides were mounted in buffered glycerol and examined with a Zeiss fluorescence microscope.

An indirect IF test was used to detect antibodies in deer sera. Our test, modified from that of Philip et al. (1976), was evaluated with antisera produced in 21- to 28-day-old Swiss mice (Anderson et al., 1983). Intravenous inoculations of 0.3–0.5 ml of Kelly's medium containing $\geq$16,600 spirochetes/ml were given to mice on days 0 and 7. Individual mice were exsanguinated on days 14–18, and sera of chal-

TABLE 1. Average numbers of adult *Ixodes dammini* removed from white-tailed deer in Connecticut during 1982.

Check stations	Total animals examined	No. (%) with ticks	No. of *I. dammini*	
			Males $\bar{x} \pm SD$	Females $\bar{x} \pm SD$
Litchfield[a]	17	0	0	0
Sharon[a]	21	0	0	0
East Haddam	52	32 (62)	3.2 ± 2.7	3.7 ± 2.6
East Lyme	81	66 (82)	5.0 ± 3.4	4.8 ± 2.7
Haddam	105	45 (43)	2.0 ± 1.0	2.3 ± 1.3
Voluntown	85	45 (53)	1.8 ± 0.9	1.7 ± 1.2
Totals	361	188 (52)		

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

lenged and uninoculated (control) animals were screened against a series of spirochete isolates in reciprocal cross tests. The sensitivity and reproducibility of indirect IF procedures were further demonstrated with acute and convalescent sera of persons having a history of Lyme disease. Spirochete antigens, isolated from *I. dammini* or wild mammals and grown in fortified Kelly's medium (Burgdorfer et al., 1982), included strains from East Haddam, Connecticut (#2356) and Prudence Island, Rhode Island (#2881). Isolation procedures, preparation of working antigens, serum titrations, and source of FITC-labeled rabbit antisera to white-tailed deer have been previously reported (Burgdorfer et al., 1982; Anderson et al., 1983; Magnarelli et al., 1984). Reactions ≥1:64 were considered positive; titration endpoints refer to the highest serum dilutions for which there was definite fluorescence of spirochetes. Assays included antigen, conjugate, and negative and positive serum controls.

Positive sera from Haddam and Voluntown were re-tested against both strains of antigens to compare reactivity. In this trial, glass microscope slides contained two rows of five antigen preparations of each strain. Diluted sera were drawn from microtiter plates and divided so that aliquots of each dilution could be placed over respective spots of reagent in each series. Slides were processed as before by indirect IF, and endpoints were determined to confirm reproducibility and to assess variability.

RESULTS

Male and female *I. dammini* were removed from deer brought to check stations at East Haddam, East Lyme, Haddam, and Voluntown. Of the 323 animals examined at these sites, 188 (58%) had ticks (Table 1). Parasitism ranged from 43% at Haddam to 82% at East Lyme. The average numbers of *I. dammini* adults removed from hosts were highest at East Lyme. Examinations of 38 deer from Litchfield and Sharon revealed no *I. dammini*. Voucher specimens of these ticks have been deposited in the Peabody Museum of Natural History, Yale University, New Haven, Connecticut 06511, USA.

Spirochetes were observed in 18 of 133 (14%) ticks collected during 1982. Two female ticks from East Lyme and Haddam had spirochetes in hemolymph and midgut preparations (Table 2). An additional 16 ticks, negative by direct FA staining of hemolymph, harbored these bacteria in their midguts; infection rates of 10–18% were recorded at four sites in Connecticut.

Indirect IF tests revealed antibodies to spirochetes in 93 (28%) of the 332 sera assayed (Table 3). Positive sera were obtained from four endemic areas in Connecticut. Rates ranged between 27% and 39%, and antibody titers for 65 samples (70% of total positives) were within 1:64–512. Samples collected in Litchfield and Sharon were negative.

Comparative studies of 42 positive sera from Haddam and Voluntown showed little difference in reactivity against strains of antigens isolated in East Haddam and

TABLE 2. Total *Ixodes dammini* examined by hemolymph and fluorescent antibody tests for spirochetes.

Check stations	Total ticks tested ♂	Total ticks tested ♀	Number ticks FA positive: Hemolymph ♂	Hemolymph ♀	Gut smears ♂	Gut smears ♀	% FA positive*
East Haddam	12	8	0	0	0	2	10
East Lyme	52	11	0	1	5	3	13
Haddam	10	12	0	1	2	1	14
Voluntown	14	14	0	0	1	4	18
Totals	88	45	0	2	8	10	14

* % = Total positive by FA of midgut tissues ÷ total ticks dissected from each station × 100.

TABLE 3. Total sera of white-tailed deer analyzed for antibodies to spirochetes in Connecticut during 1982.

Check stations	Total sera tested	No. (%) positive	No. samples at reciprocal titration endpoints: 64–128	256–512	1,024–2,048	4,096
Litchfield	17	0	—	—	—	—
Sharon	21	0	—	—	—	—
East Haddam	48	13 (27)	7	4	1	1
East Lyme	70	19 (27)	8	6	2	3
Haddam	101	39 (39)	15	10	13	1
Voluntown	75	22 (29)	11	4	6	1

Prudence Island. Titration endpoints were identical for 26 sera and were within normal test variation (2- to 4-fold differences) for an additional 14 samples (Table 4). Although two serum samples had 8-fold variation in reactivity, seropositivity was highly reproducible.

DISCUSSION

Ixodes dammini adults parasitized deer during the fall. This reaffirms earlier findings (Wallis et al., 1978; Piesman et al., 1979; Spielman et al., 1979; Anderson and Magnarelli, 1980), which showed a close ectoparasite/host relationship. In addition to deer, *I. dammini* larvae and nymphs will also feed on various small and medium-sized mammals (Carey et al., 1980, 1981; Main et al., 1982). Since earlier studies have verified spirochetes in immature *I. dammini* (Anderson et al., 1983), small as well as large vertebrates may play a role in the dissemination of infected ticks.

There is a close correlation among the distribution of infected *I. dammini*, the occurrence of human cases of Lyme disease, and the presence of seropositive deer or other mammals (Magnarelli et al., 1984) in southcentral and eastern Connecticut. In contrast, mammalian sera collected in northwestern Connecticut and in Newtown (Fig. 1) (Magnarelli et al., 1984), areas where *I. dammini* is rare and where no human cases of Lyme disease have been reported, contained no antibodies to spirochetes. Since our tick infection rates of 10–18% were lower than those reported by Burgdorfer et al. (1982) for Shelter Island, New York (61%) and by Anderson et al. (1983) for three sites in Connecticut (27–45%), there may be variation due to locale or temporal factors. Nonetheless, these results suggest that these bacteria are enzootic, and assuming that spirochetes are the etiologic agents of Lyme disease, there is potential for infections in human and wildlife populations.

Although antibodies were found in deer from four areas endemic for Lyme disease, there are probably numerous foci for

TABLE 4. Differences in serum reactivity against spirochetes isolated from *Ixodes dammini* in Connecticut or Prudence Island in 1982.

Check stations	Total positive sera tested	Differences in antibody titers to spirochetes*: None	2-fold	4-fold	8-fold
Haddam	26	16	8	1	1
Voluntown	16	10	2	3	1

* Antigens used included #2356 and #2881 isolated from *I. dammini* in East Haddam, Connecticut and Prudence Island, Rhode Island, respectively.

spirochetes in southcentral and eastern Connecticut. Immature and adult *I. dammini* might transmit these organisms to mammals during all seasons.

Medium and small-sized mammals such as raccoons, white-footed mice, and eastern chipmunks are also exposed to infected *I. dammini* (Anderson et al., 1983) and contain antibodies to spirochetes (Magnarelli et al., 1984). The pathogenic effects, if any, of spirochetes on these hosts are unknown. ECM, an important clinical marker for human illness (Steere et al., 1977), has yet to be described for these mammals. Furthermore, times of infection, immune responses, persistence of immunoglobulins, and duration of spirochetemia need clarification. Challenge studies are needed to define clinical features and to identify hosts that may serve as efficient reservoirs for infecting ticks.

Originally discovered in the digestive tracts of adult *I. dammini* (Burgdorfer et al., 1982), spirochetes are more likely to be found in these tissues than in hemolymph preparations. The hemolymph test is a valuable tool for the detection of spotted-fever group rickettsiae in ticks (Burgdorfer, 1970), but its application in the present study was limited. In fact, presence of spirochetes in the hemolymph of two ticks may have been due to inadvertent rupturing of midguts while handling specimens.

There was little difference in the reactivity of positive deer sera with two strains of spirochetes. As reported earlier (Anderson et al., 1983; Magnarelli et al., 1984), different isolates from *I. dammini* or wild mammals in the northeastern United States reacted similarly in our indirect IF tests. Since human cases of Lyme disease also occur in western states where spirochete-infected *Ixodes pacificus* abounds (Steere et al., 1978; Steere and Malawista, 1979; Burgdorfer et al., 1982), and since ECM is reported in Europe where *I. ricinus* occurs (Steere and Malawista, 1979), all three species of ticks may harbor and transmit these bacteria to humans and wildlife.

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BOOK REVIEW . . .

The Red Fox: Symposium on Behavior and Ecology, E. Zimen, ed. Dr. W. Junk B.V. Publishers, P.O. Box 13713, 2501 ES, The Hague, The Netherlands. 1980. 292 pp. $73.50 US; 140 Dutch Guilders.

The continued threat of fox rabies in Europe prompted the gathering of the leading fox biologists and rabies experts in Europe to the Saarbrücken Fox Colloquium in January 1979. Eighteen papers presented at that meeting are included in this book along with introductory and closing remarks by the editor. Two of the papers are not in English.

Fourteen papers dealing with fox biology make up 235 of the 285 pages. The final five chapters discuss the role of the fox in rabies in Europe.

Good data on fox habitat requirements are presented by H. G. Lloyd. Three chapters on food habits include one paper by D. Sequiera that has an excellent bibliography of 119 references. Population dynamics is discussed in three papers while social factors affecting reproduction is presented in the longest chapter of 52 pages. This latter paper, although detailed, suggests more questions than answers. Two chapters deal with incomplete research projects which could have been omitted.

The papers on rabies are adequate reference material for those interested in the European rabies problem. Dr. Wandelar presents a good review of the epidemiology of fox rabies with 76 references. Drs. Bogel and Moegle discuss the spread of the wildlife epizootic in Germany with the fox population dynamics in respect to rabies given for France by Drs. Artois and Andral. One paper dealing with the epizootiology and control of rabies in Central Europe suggests that rabies infection does not continue in European mustelids in the absence of fox rabies because mustelids excrete significantly less infectious virus in their saliva. A further postulate is that European hamsters are not important in the epizootiology of rabies due to their resistance to infection and lack of high infectious titers in the salivary glands. These two theories, both contrary to North American studies, make for interesting, albeit conjectural, reading.

In reading the papers, one has the feeling that studies on fox biology remain a fertile field for investigation. Since all wildlife disease studies require a team approach involving but not limited to the biologist and the health scientist, this book would be interesting to the wildlife disease student and investigator, irrespective of their interest in rabies.

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