Prevalence of the Lyme Disease Spirochete in Populations of White-Tailed Deer and White-Footed Mice

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The prevalence of the *Ixodes dammini* spirochete (IDS) in white-tailed deer (*Odocoileus virginianus*) and white-footed mice (*Peromyscus leucopus*) was studied on the eastern end of Long Island, New York. Both species commonly occur in a variety of habitats, are preferred hosts of *Ixodes dammini,* and can harbor the spirochetes in the blood. Each animal was examined for spirochetemia, tick infestation, and IDS infection rates in the ticks that were removed from it. The results obtained suggest that in winter deer can be infected by questing adult *I. dammini.* Adult ticks apparently are infected through transtadial transmission of spirochetes from subadult ticks which had fed earlier in their life history on infected animals. Deer are important hosts of adult ticks and the IDS in winter and probably are a reservoir host in other seasons. The patterns of spirochete prevalence suggest that deer and mice are reservoirs of the organism and thus are fundamental to the ecology of Lyme disease on Long Island.

INTRODUCTION

Epidemiologic evidence in the northeastern United States suggests that nymphal *Ixodes dammini* ticks transmit both *Babesia microti* [1] and the causative agent of Lyme disease [2,3] to humans. In 1981, 61 percent of adult *I. dammini* collected from an endemic area on Shelter Island, New York, harbored spirochetes [4] now accepted to be the etiologic agent of Lyme disease [5,6].

I. dammini is a three-host tick that parasitizes a wide range of avian and mammalian hosts. Even though all tick stages are found on most mammalian species, not all animals serve equally as hosts; each successive post-embryonic stage apparently prefers to feed on a progressively larger host. Larvae, abundant in late summer and early fall, commonly feed on small rodents, particularly the white-footed mouse (*Peromyscus leucopus*) [7–11]. Nymphs, numerous in spring and summer, preferentially feed on medium-sized mammals such as sciurids and raccoons, but also feed on *P. leucopus* [8–11]. Adult ticks feed on larger mammals, principally white-tailed

deer (*Odocoileus virginianus*), in the fall, early winter, and spring [11–13]. Deer appear to be an important host to all tick stages, as each stage has been reported to feed on deer during its appropriate activity period [11–13]. All stages have been observed to feed on humans [13].

In 1982, *I. dammini* spirochetes (IDS) were isolated from blood of *P. leucopus* and *O. virginianus* collected from endemic areas of Shelter Island that yielded the original tick spirochete isolates [11]. Engorged larval ticks and unengorged nymphal ticks recovered from these animals harbored spirochetes [11].

P. leucopus demonstrates limited movement in relation to larger species, is ubiquitous, and is probably the most abundant rodent in all seral forest stages on Long Island [14]. White-tailed deer are abundant in Lyme disease-endemic areas on the eastern end of Long Island in habitats overlapping those of *P. leucopus*. To more fully elucidate the natural history of Lyme disease, studies of these two animal species were initiated. The role of white-tailed deer is examined principally in this report.

MATERIALS AND METHODS

Deer Samples

Deer were sampled from endemic areas on eastern Long Island during hunting seasons (January), by road-kills, and by special collection permit from November 1981 through January 1983. During the hunting season, hunters are required to bring harvested deer to check stations operated by the State Department of Environmental Conservation. Demographic and select physiological, nutritional, and reproductive parameters are recorded to determine the size and condition of the herd. Deer must be presented in a non-eviscerated condition to obtain these data; evisceration and sampling are performed on site by trained personnel.

Following incision and retraction, the pleural cavity was examined for wounds or other internal damage. If no obvious damage was detected, citrated and clotted blood samples were aseptically aspirated from the aortic artery. Blood was collected in appropriate sterile vacuum tubes following thorough rinsing of the artery with 70 percent ethanol. In moderately damaged deer, aseptic cardiac puncture was attempted, while in severely damaged individuals blood was collected from the cavity with a syringe. Four thin whole blood smears were made from each animal examined.

Spirochete Detection

An aliquot (0.1 ml) of sterile citrated blood was transferred immediately from the collection tube into another sterile vacuum tube containing 8 ml of modified Kelly's medium [15]. Sterile transfer of blood to culture medium in the field was performed with a tuberculin syringe after stoppers on both tubes had been sterilized with 70 percent ethanol. Additionally, 0.1 ml of citrated blood was inoculated into 8 ml of medium in the laboratory under a laminar flow hood no more than 12 hours after field collection. All cultures were maintained at $33 \pm 2°C$, and examined every other day for spirochete growth by darkfield microscopy over a six-week period. Culturing was not attempted on deer collected prior to isolation of the IDS from ticks in June 1982 [4].

Plain microhematocrit (capillary) tubes were filled with citrated blood and centrifuged at 37,500 g for two minutes. Tubes were subsequently cut at the interface of the buffy coat and erythrocytes. The buffy coat and plasma were placed on a slide

and examined by darkfield microscopy for spirochetes. A similar technique for detection of leptospires with a lower limit of sensitivity of 10^3–10^4 leptospires per ml of blood has been described [16].

All blood smears were fixed for ten minutes in acetone-free methanol. One smear was stained with Giemsa blood stain and another with fluorescein isothiocyanate conjugated (FITC) rabbit anti-IDS serum (37°C for forty-five minutes) [11]. Giesma-stained smears were initially examined by oil immersion for *B. microti,* spirochetes, and blood trypanosomes. Subsequently, a cover slip was placed on the oil-covered smear and the slide examined by darkfield microscopy; spirochetes were easily discernable as white or yellow. Fluorescein-stained smears were examined under an ultraviolet microscope for spirochetes.

Spirochetes detected by any method were identified as the IDS by reactivity to FITC rabbit antisera to the IDS in direct immunofluorescence tests [11].

Antibody Determinations

Serum from clotted blood was used for antibody determinations to the IDS by indirect immunofluorescence (IFA). Antigen preparation and test procedures were identical to what has been described previously [5]. Commercially prepared rabbit anti-deer (Miles Laboratories, Elkhart, IN) was labeled with fluorescein isothiocyanate by W. Burgdorfer (Rocky Mountain Laboratories, Hamilton MT), diluted with phosphate-buffered saline (pH 7.4) to 1:20, and used as conjugate. Endpoints were defined as the highest twofold serial dilution at which all spirochetes fluoresced dimly. Positive control sera were obtained from highly reactive Long Island deer and negative controls from deer collected in non-endemic areas of upstate New York.

Tick Examination

Individual deer were examined for ticks on a single specified body region (head and neck; chest and stomach; back; legs and inguinal area) for ten minutes. Ticks were detected by examination and manipulation of the fur with forceps and a wide-toothed comb. Ticks from each animal were removed with forceps and placed in a vial containing a strip of filter paper premoistened with distilled water. Subsequently, ticks were separated according to sex and degree of blood engorgement and individually examined for IDS by methods previously described [11].

Questing Tick Collections

Questing adult ticks were collected by flagging vegetation along trails for a premeasured distance and from the investigator's clothing while randomly walking through an area for thirty minutes.

RESULTS

From November 1981 through January 1983, 110 white-tailed deer were examined from endemic areas of Lyme disease on eastern Long Island. One hundred and five deer were sampled during hunting seasons. Of these 73 percent were from Shelter Island, 16 percent from North Haven (separated from Shelter Island by less than one-half mile of water), and the remaining 11 percent from widely dispersed areas along the south shore of Long Island. Three road-kill deer were sampled in November (1981 and 1982). One road-kill female fawn and one 27-month-old male (shot) were sampled in August 1982.

Deer were categorized into four age classes: fawns (less than 12 months), yearlings

TABLE 1
Number (and Percentage) of White-Tailed Deer
Examined for Spirochetes from 1981–1983,
by Age and Sex

	Sex		
Age	Male (%)	Female (%)	Total (%)
Fawn (<12 months)	15 (50.0)	15 (50.0)	30 (27.3)
Yearling (13–23 months)	21 (58.3)	15 (41.7)	36 (32.7)
Young Adult (24–35 months)	14 (58.3)	10 (41.7)	24 (21.8)
Adult (36 + months)	7 (35.0)	13 (65.0)	20 (18.2)
Total	57 (51.8)	53 (48.2)	110

(12–23 months), young adult (24–35 months), and adults (36 months and older). Approximately equal numbers of male and female deer were examined with a slightly higher percentage of young males than females and almost twice as many adult females as males (Table 1). The sample size of each age class was proportional to its occurrence in natural populations as determined by herd management programs [Knoch H, NY State Department of Environmental Conservation: personal communication].

Spirochetes were detected in 32.4 percent (16 males and 19 females) of the 108 deer sampled in November and January (winter). Of 56 total males 28.6 percent and of 52 total females, 36.5 percent were spirochetemic during the winter (Table 2). Table 2 indicates infection was statistically independent of deer sex ($G = .78$, 1 df, $p > .1$) and age ($G = 3.34$, 3 df, $p > .1$). Yearlings showed a statistically nonsignificant higher percentage of infection when compared to other age classes; the majority of spirochetemic deer were less than three years old. As previously reported [11]

TABLE 2
Percentage (and Sample Size) of 108 White-Tailed Deer
Sampled in November and January[a] (1981–1983) That Had
Blood Spirochetes, by Sex[b] and Age[c]

	Sex		
Age	Male (%)	Female (%)	Total (%)
Fawn	26.7 (15)	35.7 (14)	31.0 (29)
Yearling	35.0 (20)	53.3 (15)	42.9 (35)
Young Adult	35.7 (14)	20.0 (10)	29.2 (24)
Adult	0 (7)	30.8 (13)	20.0 (20)
Total	28.6 (56)	36.5 (52)	32.4 (108)

[a] Values do not include a spirochetemic fawn from August [11].
[b] ($G =0.78$, 1 df, $p > 0.1$)
[c] ($G =3.34$, 3 df, $p > 0.1$)

TABLE 3

Antibody Response of White-Tailed Deer ($n = 83$)
to the *Ixodes dammini* Spirochete in Spirochetemic
and Non-Spirochetemic Deer

Reciprocal Titer	Spirochetes Detected	
	Yes	No
1	3	5
16	5	9
32	8	15
64	9	13
128	0	11
256	0	5

1. Sample based on deer collected in all seasons
from 1981 through 1983

2. Distribution of reciprocal titers was not significantly different from a normal distribution
($D_{max} = 0.124$, 80 df, $p > 0.2$).

spirochetes were cultured from aortic blood of a two-month-old female fawn killed in early August; a 27-month-old male sampled in mid-August was non-spirochetemic.

No *B. microti* were found in the erythrocytes of any deer. Trypanosomes (presumably *Trypanosoma thieleri*) were detected in 32 deer, six of which were concurrently infected with spirochetes.

Eighty-one winter deer and two deer sampled in August were tested for antibody responses to the IDS. Reciprocal titer values ranged from 1–256 and were approximately normally distributed over the total sample (Table 3). Deer with demonstrable spirochetemia never expressed titers greater than 1:64, while non-infected deer possessed titers as high as 1:256 (Table 3).

Table 4 provides the geometric mean titer values for the 73 seropositive winter-collected deer expressed as a function of infection and deer age. Titers of these deer were normalized by log transformation to permit analysis of variance. Mean titers were highest in non-infected deer and in infected adults. Values were somewhat lower in infected deer in the three youngest age classes. Of the deer sampled in August, the infected fawn possessed a titer of 1:16 while the non-infected young adult possessed a titer of 1:128.

A total of 71 deer (67 from January and two each from November and August) were examined for ticks. Of all deer examined, 53 (74.6 percent) were infested with *I. dammini;* no other tick species were recovered. Deer harvested in January pos-

TABLE 4

Geometric Mean Reciprocal Titer (and Sample Size)
for 73 Seropositive Deer by Age and Spirochete Status

Age	Spirochetes Detected	
	Yes	No
Fawn	35 (7)	51 (16)
Yearling	41 (6)	51 (13)
Young Adult	27 (4)	58 (12)
Adult	54 (4)	43 (11)

sessed adult ticks only. One of two deer sampled in November was infested with six engorged nymphs and 19 male and nine female ticks. No adult ticks were recovered from deer sampled in August; a fawn was infested with 22 larvae and three nymphs [11] while a young adult male had 310 larval *I. dammini*. Regression analysis indicated no significant relationship between the number of ticks recovered and time elapsed (up to nine hours) since death of the host ($N = 69$, $Y = -0.46 X + 6.99$, $r^2 = 0.01$). Ninety-four percent of all deer were examined prior to five hours after kill and loss of ticks was considered to be negligible.

A total of 415 adult ticks were collected; 233 were males ($\bar{x} = 3.38 \pm 5.03$ SD) and 182 females ($\bar{x} = 2.64 \pm 3.36$). Although several males were attached to the host, the majority were seen crawling in the fur or copulating with females. Forty-one non-engorged female ticks ($\bar{x} = 0.59 \pm 1.33$) and 141 females ($\bar{x} = 2.04 \pm 3.15$) in various degrees of blood engorgement were removed from deer. Total mean tick burden for the entire deer population sampled was 6.10 ± 8.18.

No statistical difference in total tick burden due to deer sex or age class was noted. Male ticks were significantly more numerous on male deer ($\bar{x} = 2.8$, $n = 33$) than on female deer ($\bar{x} = 1.7$, $n = 36$; $F = 4.8$, $p < .05$), but age of the deer was nonsignificant in explaining the variance in numbers of male ticks.

Deer age was a non-significant variance component of spirochete infection in any of the classes of ticks, i.e., males, engorged females, and non-engorged females. The mean percentage of infected ticks pooled across all age classes was three times greater in non-engorged females (79 percent, $n = 20$) than in males (25 percent, $n = 150$); or engorged females (26 percent, $n = 121$); tick infection thus depended on tick class ($G = 23.69$, 2 df, $p < .005$).

One of 53 larval *I. dammini,* and one of three nymphs collected from the two deer sampled in August were infected; only engorged subadults were infected and these were removed from the spirochetemic deer [11]. No spirochete-infected ticks were recovered from a non-spirochetemic deer collected in November 1982. Ticks from a spirochetemic deer (November 1981) were not examined for the IDS.

Adult questing ticks show bimodal seasonality with greatest activity from October through December and to a lesser extent in April and May (Table 5). Questing individuals were encountered sporadically on particularly mild days in January, February, and March. Sixty-one percent of free-ranging adult ticks collected from Shelter Island in late September and October 1981 were found to harbor the IDS [4].

Monthly infection rates for 535 (273 female and 262 male) free living adult ticks in 1982 indicate that the highest spirochete infection occurs during the fall questing peak (Table 5). A slightly greater percentage of all male ticks (24.9 percent) collected contained spirochetes than did female ticks (22.2 percent).

TABLE 5
Number (and Percentage) of Free-Ranging *Ixodes dammini* That Were Infected,
by Collection Month and Tick Sex

Month	Total ticks Collected	Number Infected (%)	Tick Sex	
			Male (%)	Female (%)
October	175	73 (41.7)	41 (23.4)	32 (18.3)
November	234	130 (55.6)	62 (26.5)	68 (29.1)
December	81	35 (43.2)	22 (27.2)	13 (16.0)
April	27	9 (33.3)	6 (22.2)	3 (11.1)
May	18	5 (27.8)	2 (11.1)	3 (16.7)
Total	535	252 (47.1)	133	119

DISCUSSION

In 1982 the IDS was isolated from the blood of white-tailed deer and white-footed mice [11]. Deer were spirochetemic during the summer, fall, and winter and implicated as reservoirs of infection. The current study reaffirms that deer are spirochetemic in the fall and winter in that 32 percent harbored the organism. Failure to detect *B. microti* within erythrocytes supports previous findings that deer do not function as reservoirs of this organism [12].

Serum antibody to the IDS was detected in 88 percent of the deer tested and was approximately normally distributed in the population, indicating that the majority of deer had been exposed to the spirochete. Without knowing the temporal associations of spirochete exposure or infection, it appears that antibody titers of 1:128 or greater may inhibit spirochetemia since spirochetemic deer never had titers of greater than 1:64, whereas non-spirochetemic animals had levels as high as 1:256. The duration of antibody in detectable levels and whether or not the spirochete becomes cryptic in another organ system during periods of titer are unknown.

Examination of only seropositive deer suggests higher mean titers tended to occur in non-infected deer of all age classes and infected adults. The former suggests spirochete inhibition whereas the latter may indicate cummulative exposure to the parasite.

Greater infestations of *I. dammini* on bucks than does, and on yearlings and adults compared to fawns has been reported in Connecticut [13]. We observed no difference in total tick burden due to deer sex or age class; however, male ticks were more numerous on male deer. These discrepancies may be due to temporal or host behavioral responses affecting tick acquisition.

In this study, spirochetes were detected in 41.7–55.6 percent of fall questing adults and in 25–79 percent of the various adult tick classes removed from deer. Since the highest mean percentage of infection was found in non-engorged ticks removed from deer, we suggest that these ticks were recently acquired by the deer and capable of transmitting the IDS to them in winter. Transmission of the spirochete by adult *I. dammini* to laboratory rabbits [17,18] and to humans [19] further supports the latter hypothesis. Although transtadial transmission of the IDS from subadults to both adult sexes has been observed in our laboratory [Bosler EM: unpublished data], male tick feeding behavior and subsequent transmission require investigation.

P. leucopus with spirochetemia and attached spirochete-infected subadult ticks have been reported from Shelter Island [11]; spirochetemic mice have been observed from May (36 percent) through November (4 percent) [Bosler EM: unpublished data]. Thirty-seven percent of 306 ticks removed from mice were found to be infected in preliminary studies [11]. To date, 99 percent of all infected subadult ticks collected from small rodents were recovered from *P. leucopus;* 39 percent of all white-footed mice were infested with infected immature ticks [11; Bosler, EM: unpublished data].

We suggest that both white-tailed deer and white-footed mice act as reservoirs of Lyme disease on Long Island, since both animals commonly occur in a variety of habitats [20] in areas of endemic Lyme disease, are principal hosts of spirochete-infected *I. dammini,* and are spirochetemic [11]. Deer are spirochetemic in summer, fall, and winter whereas mice are found infected during the spring, summer, and fall [11; Bosler EM: unpublished data]. Spirochete-infected immature *I. dammini* have been removed from both species of animals during the summer and fall, whereas infected adult ticks were only recovered from deer (fall and winter).

Transovarial transmission of the IDS has been established for naturally infected *I.*

ricinus, I. pacificus [11,21] and suggested for *I. dammini* [11]. However, transovarial transmission of generalized infection to filial ticks is unlikely [19]. Most subadults probably acquire the IDS by feeding on a spirochetemic host. We suggest that *P. leucopus* is the major small rodent reservoir for infection of larval ticks since it is the most abundant small rodent on Long Island [14,20], and is a consistent reservoir of IDS during the period of immature activity [11; Bosler EM: unpublished data]. Although few deer have been sampled during periods of subadult activity, the occurrence of infected subadults on infected deer has been established in this report. This suggests that ticks may be infected by feeding on spirochetemic deer in the summer. The role of deer as winter reservoirs of infection depends upon the degree to which the adult ticks successfully transmit the IDS to their offspring. However, as stated previously, it is unlikely that transovarial transmission plays a major role in the life history of the IDS. Controlled transmission studies are needed to fully understand the roles of deer and mice in the ecology of Lyme disease.

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