PREVALENCE OF THE LYME DISEASE SPIROCHETE, BORRELIA BURGDORFERI, IN DEER TICKS (IXODES DAMMINI) COLLECTED FROM WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN SAINT CROIX STATE PARK, MINNESOTA

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PREVALENCE OF THE LYME DISEASE SPIROCHETE, *BORRELIA BURGDORFERI*, IN DEER TICKS (*IXODES DAMMINI*) COLLECTED FROM WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN SAINT CROIX STATE PARK, MINNESOTA

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ABSTRACT: During a special two-day hunt (11, 12 November 1989) in Saint Croix State Park, Minnesota (USA), one side of the neck for each of 146 white-tailed deer (*Odocoileus virginianus*) was examined for ticks. Of the 5,442 ticks collected, 90% (4,893) were the winter tick, *Dermacentor albipictus*, and 10% (549) were the deer tick, *Ixodes dammini*, the primary vector of the causative agent of Lyme disease, *Borrelia burgdorferi*. Adult males had the greatest frequency of infestation of either *D. albipictus* (100%) or *I. dammini* (88%) and had on average more ticks, compared to other deer. Based on an examination of midgut material from 435 *I. dammini* by polyclonal antibody analysis, spirochetes were observed in 22% of the ticks. Species-specific monoclonal antibody analysis of the spirochetes confirmed that the bacteria were *B. burgdorferi*.

Key words: Lyme disease, *Borrelia burgdorferi*, *Ixodes dammini*, white-tailed deer, *Odocoileus virginianus*, Minnesota.

INTRODUCTION

The spirochete that causes Lyme disease (Burgdorfer et al., 1982), *Borrelia burgdorferi* (Hyde and Johnson, 1984; Johnson et al., 1984), is found primarily in ticks of the *Ixodes ricinus* complex. This bacterium has been identified in *I. dammini* in the northcentral and northeastern USA (Burgdorfer et al., 1982; Anderson et al., 1983; Anderson et al., 1987; Drew et al., 1988), in *I. scapularis* in the southern and southeastern USA (Magnarelli et al., 1986; Luckhart et al., 1991; Teltow et al., 1991), in *I. pacificus* in the western USA (Burgdorfer et al., 1985), in *I. ricinus* in Europe (Barbour et al., 1983a), and in *I. persulcatus* in Eurasia (Ai et al., 1988; Korenberg et al., 1989) and Japan (Miyamoto et al., 1991). Spirochetes also have been detected less frequently in the ticks *Amblyomma americanum* (Schulze et al., 1984; Magnarelli et al., 1986; Luckhart et al., 1991; Teltow et al., 1991), *A. maculatum* (Teltow et al., 1991), *Haemophysalis leporispalustris* (Lane and Burgdorfer, 1988), *Dermacentor variabilis* (Anderson et al., 1985), and *D. albipictus* (Magnarelli et al., 1986); the role of these latter ticks as vectors has not been established. The spirochete has been cultured from one species of passarine bird (*Catharus fuscescens*) (Anderson et al., 1986) and from many different wild mammals, including the white-footed mouse (*Peromyscus leucopus*) (Anderson et al., 1985; Loken et al., 1985). White-tailed deer (*Odocoileus virginianus*), the primary host of adult *I. dammini*, are seemingly not good reservoirs for *B. burgdorferi* (Loken et al., 1985; Telford et al., 1988), although spirochetes, presumably *B. burgdorferi*, have been observed in deer blood samples (Bosler et al., 1983) and in the midgut of a single female *D. albipictus*, a one-host tick, collected from a white-tailed deer (Magnarelli et al., 1986).

In humans, Lyme disease causes musculoskeletal, cardiac, and central nervous system disorders, often preceded early in the disease by the pathognomic skin lesion, erythema migrans, at the site of the tick bite (Steere et al., 1983; Steere, 1989). The effects of Lyme disease on domestic animals, especially dogs, have been docu-

mented and include symptoms such as lameness, loss of appetite, and heart blockage (Lissman et al., 1984; Magnarelli et al., 1985). Adverse effects of *B. burgdorferi* infection in wild animals have not been reported or observed.

Although workers have revealed large fluctuations in infection rates of *B. burgdorferi* in *I. dammini* in the northeastern USA, ranging from 12% to 100% (Anderson and Magnarelli, 1983; Burgdorfer and Gage, 1986), relatively little has been reported on the prevalence of the Lyme disease spirochete in *I. dammini* in Minnesota (USA) where the environmental conditions are different (i.e., longer, colder winters) than those observed in the northeastern states. Drew et al. (1988) reported a prevalence of the spirochete in adult *I. dammini* of 10%. Others (Anderson et al., 1987; Callister et al., 1988; Callister et al., 1991) have shown that between 35 and 75% of *I. dammini* in Wisconsin (USA) were infected with the spirochete. In the current study we determined the number of ticks at a specific site (i.e., one side of the neck) on white-tailed deer and the prevalence of *B. burgdorferi* in *I. dammini* ticks in the geographic area in Minnesota.

MATERIALS AND METHODS

Ticks were collected from white-tailed deer during a two day hunt on 11 and 12 November 1989 at St. Croix State Park (45°57'N, 92°37'W) in Pine County in east-central Minnesota. St. Croix State Park, a widely used recreational area, is Minnesota's largest state park, covering 13,760 hectares. It is located along the western bank of the St. Croix River which forms a portion of the boundary between Minnesota and Wisconsin. All deer killed during the hunt were brought to a single registration station where 146 of the 406 harvested deer were randomly selected for examination. Adult *I. dammini* are concentrated on the neck of white-tailed deer (Watson and Anderson, 1976). Therefore, we restricted our examination and collection of ticks to one side of the neck from the base of the head to the top of the shoulder from each deer. Examination, on average, occurred 4.7 hr after death and collection time ranged from 5 to 90 min depending upon the number of examiners per deer and the number of ticks found. Ticks were placed in humid containers, chilled, identified to life stage and species (Sonenshine, 1979; Keirans and Litwak, 1989), and frozen at −20 C. In addition to collecting ticks, information on each examined deer was obtained regarding age, as determined by dental patterns and tooth wear (Severinghaus, 1949), weight, sex, time of kill, and time of examination.

Portions of the midgut of *I. dammini* ticks were examined for the presence of *B. burgdorferi* by indirect immunofluorescence assay (IFA), as follows. Cuts were made at the caudal end of each tick to expose the midgut region. A small amount of midgut material was removed, smeared onto glass microscope slides, and air-dried. The material was fixed in acetone for 15 min and air-dried. Midgut material was overlaid for 30 min in a moist chamber at 37 C with 3 µl of hyperimmune rabbit anti-*B. burgdorferi* strain 297 serum (R. C. Johnson, University of Minnesota, Minneapolis, Minnesota; IFA titer of 1:1,024) diluted 10-fold in 0.01 M phosphate-buffered saline (PBS), pH 7.4. After being rinsed in PBS and allowed to air dry, the midgut material was overlaid with 3 µl of fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit IgG (Cappel/Organon Teknika, Durham, North Carolina, USA) and incubated for 30 min in a moist chamber at 37 C. Slides were rinsed in PBS and then distilled water, air-dried, mounted in PBS-buffered glycerol, and examined with a Leica Laborlux 12 fluorescence microscope (E. Leitz, Rockleigh, New Jersey, USA). Positive control slides prepared from cultured *B. burgdorferi* strain 297 were included with each set of midgut slides.

Tick midgut material also was examined for the presence of *B. burgdorferi* by IFA using monoclonal antibody H5332 that reacts with the species-specific 31 kDa outer surface protein (OspA) of *B. burgdorferi* (Barbour et al., 1983b). The differences in the polyclonal and monoclonal antibody procedures are as follows: 3 µl of H5332 hybridoma culture supernatant (A. G. Barbour, University of Texas Health Sciences Center, San Antonio, Texas, USA) were used as the primary antibody to overlay tick midgut material and FITC conjugated goat anti-mouse IgG (Cappel/Organon Teknika) was used as the labelled secondary antibody.

RESULTS

On examining of one side of the neck of 146 deer, we collected, 5,442 ticks, of which approximately 90% were *D. albipictus* and 10% were *I. dammini* (Table 1). *Dermacentor albipictus* larvae, nymphs, and adults were observed, while

TABLE 1. Ticks collected from one side of the neck of 146 white-tailed deer in Saint Croix State Park, Minnesota, November, 1989.

Tick species	Number larvae	Number nymphs	Adults			Grand total
			Male	Female	Total	
Ixodes dammini	0	0	390 (7.2)[a]	159 (2.9)	549 (10.1)	549 (10.1)
Dermacentor albipictus	110 (2.0)	2,125 (39.0)	1,951 (35.9)	707 (13.0)	2,658 (48.8)	4,893 (89.9)

[a] Number (percent) of all 5,442 ticks collected.

only adult ticks of *I. dammini* were found. The ratio of *I. dammini* male to female ticks and *D. albipictus* male to female ticks was 2.5 to 1 and 2.8 to 1, respectively. (Representative specimens of *D. albipictus* and *I. dammini* were deposited in the University of Minnesota Insect Collection, Department of Entomology, St. Paul, Minnesota. Accession numbers were not assigned.)

Ticks were found on 93% of the 146 deer examined (Table 2). *Dermacentor albipictus* was found on 90% and *I. dammini* was found on 56% of the deer. Adult male deer had the greatest prevalence for both *D. albipictus* (100%) and *I. dammini* (88%). Adult female deer, although heavily infested with *D. albipictus* (86%), had a relatively low prevalence of *I. dammini* (38%). Yearlings and fawns had high prevalences of *D. albipictus* while *I. dammini* was found on only one fawn of each sex.

Adult male deer had a mean of 62.6 ticks per side of the neck per deer, the largest number of ticks of any group, while fawn females had the smallest mean number of ticks at 11.0 ticks per side of the neck (Fig. 1). The mean number of ticks found per side of the neck for all 146 deer was 37.3.

The mean number of *D. albipictus* (Fig. 2) is similar to that seen for combined ticks (Fig. 1). The mean number of *D. albipictus* per side of the neck per deer in adult males, fawn females, and all deer combined was 54.2, 10.6, and 33.5, respectively.

The mean number of *I. dammini* (Fig. 3) was lower than that seen for *D. albipictus* in all age groups for both sexes of deer (Fig. 2). The greatest mean number of ticks was carried by adult males with 8.3 *I. dammini*. Male and female fawns had the smallest mean tick burdens with only 0.1 and 0.4 *I. dammini* per side of the neck per deer, respectively. The mean number of *I. dammini* for all deer was 3.8.

Based on an analysis of midgut material from 435 of the 549 *I. dammini* ticks, 22% contained spirochetes that reacted positively with rabbit polyclonal antibodies against *B. burgdorferi* (Table 3). A random check of ticks positive for *B. burgdorferi* by polyclonal antibody analysis was done using monoclonal antibody H5332. Results

TABLE 2. Ticks collected from one side of the neck from different sex and age groups of 146 white-tailed deer in Saint Croix State Park, Minnesota, November, 1989.

Tick species	All deer (146)[a]	Males			Females		
		Adult (51)	Yearling (18)	Fawn (14)	Adult (42)	Yearling (13)	Fawn (8)
I. dammini only	81 (55.5)[b]	45 (88.2)	14 (77.8)	1 (7.1)	16 (38.1)	4 (30.8)	1 (12.5)
D. albipictus only	132 (90.4)	51 (100)	17 (94.4)	11 (78.6)	36 (85.7)	11 (84.6)	6 (75.0)
Dermacentor albipictus or *Ixodes dammini*	136 (93.2)	51 (100)	17 (94.4)	12 (85.7)	39 (92.9)	11 (84.6)	6 (75.0)

[a] Total number of deer surveyed in each category (given in parentheses).
[b] Total number (percent) of deer within the category infested with ticks.

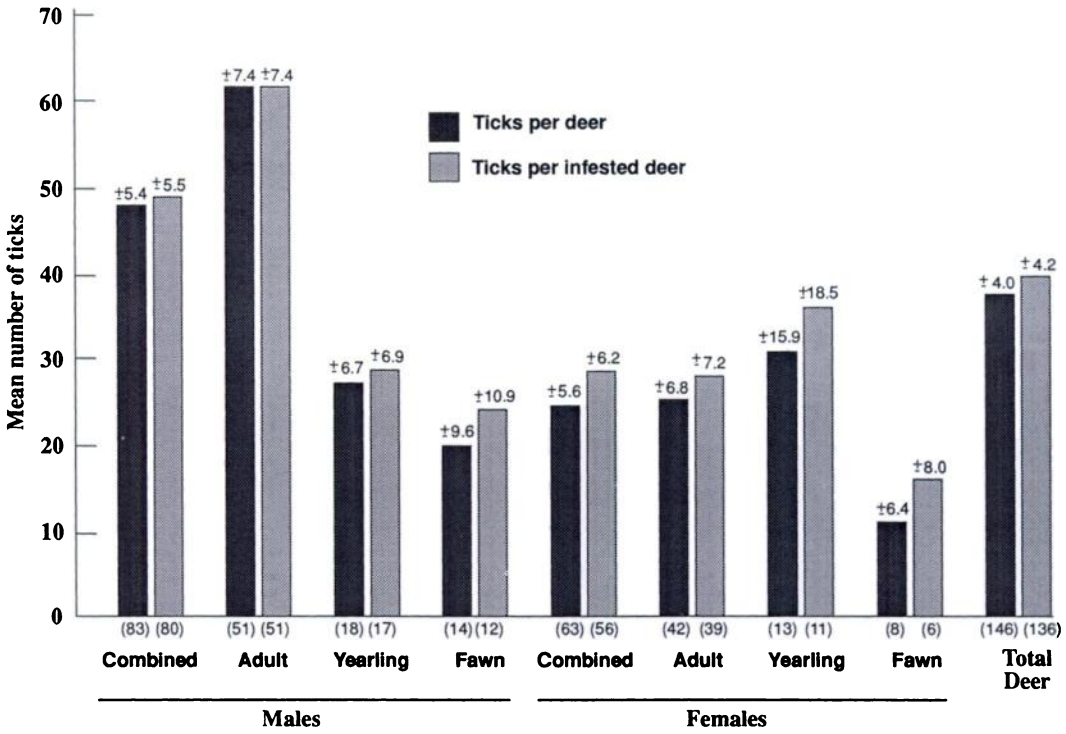

FIGURE 1. The mean number of ticks found on one side of the neck of deer grouped by sex and age of the deer. The dark bars represent the mean number of ticks found on all deer within a group. The data shown by the lighter bars exclude deer without ticks. The number in parentheses below each bar is the number of deer surveyed. The number above each bar is the standard error for each set of data.

from 95 midgut smears prepared as a second set of smears during dissection of the ticks confirmed the presence of *B. burgdorferi*. The spirochete prevalence in male and female *I. dammini* was 23% and 21%, respectively.

DISCUSSION

This is the first known attempt to collect large numbers of *I. dammini* and *D. albipictus* from white-tailed deer in Minnesota. Davis et al. (1984) evaluated one side of the neck of white-tailed deer for ticks in Wisconsin during late November in 1979 and 1981. In northwest Wisconsin, an area geographically close to our study site, they found that *I. dammini* was present on 23% of all deer in 1979 and 64% in 1981; at our study site, *I. dammini* was present on 56% of all deer. The mean number of *I. dammini* per side of the neck per deer in Wisconsin in 1979 and 1981 was 0.6 and 2.9, respectively, and the mean number of *I. dammini* per side of the neck per infested deer in 1979 and 1981 was 2.6 and 4.5, respectively (Davis et al., 1984). At our site there were on average 3.8 *I. dammini* per side of the neck on all deer and 6.8 *I. dammini* per side of the neck on infested deer. The ratio of *I. dammini* to *D. albipictus* in Wisconsin deer in 1979 and 1981 was 0.09:1 and 2.53:1, respectively (Davis et al., 1984), and at our site the ratio was 0.11:1. The similarities in the data from each state are not surprising given the relatively close geographic proximity of the two study sites. Variations in the number of ticks from year to year can be due to the many environmental factors that may influence tick populations.

In Connecticut (USA) during late November through December, 1977 to 1980, Main et al. (1981) determined, probably by whole body examination, that white-

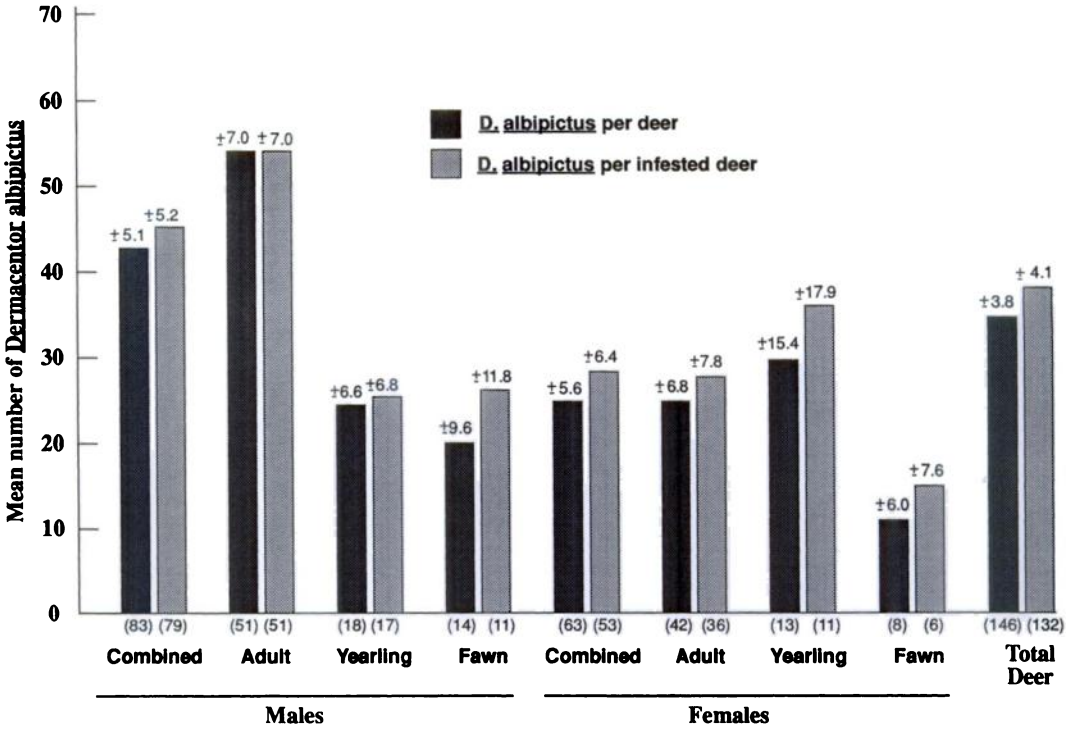

FIGURE 2. The mean number of *Dermacentor albipictus* found on one side of the neck of deer grouped by sex and age. Explanation of the dark and lighter bars and the numbers above and below the bars is given in Figure 1.

tailed males were more often infested with either adult *I. dammini* or *D. albipictus* than females, and that males had larger tick burdens than females. By examining the head, neck, shoulders, and ventral surface of white-tailed deer during the spring and fall of 1985 and 1986 in Seatuck National Wildlife Refuge in New York (USA), Wilson et al. (1990) showed also that males had larger burdens of adult *I. dammini* than females. Although our examination was limited to only one side of the neck, our results were similar. We observed that adult males were more often infested with either species of tick (Table 2) and that these deer carried the largest number of ticks (Figs. 1, 2, and 3). Adult male deer had on average approximately five times more *I. dammini* than did adult females. It also is of interest that in the current study there seemed to be a correlation of tick prevalence with age of deer. It appeared that as the deer grow older, they were more often infested with *I. dammini*. Based on a Chi-square test (Rosner, 1990), there was a significant ($P < 0.005$) difference in prevalence of *I. dammini* between fawns and older deer. Similar results were observed in Connecticut (USA) where yearlings and adults were more often infested with either *I. dammini* or *D. albipictus* and carried larger burdens than did fawns (Main et al., 1981). As suggested by Main et al. (1981), behavioral characteristics of deer that provide for increased chances of contact with questing ticks may explain the larger prevalence of ticks on older deer and the greater number of ticks found on mature males.

The 2.5 to 1 ratio of male to female *I. dammini* probably reflected a decreasing availability of female *I. dammini* at the time of sampling. In Connecticut the number of male *I. dammini* found on deer remained relatively unchanged while the number of female *I. dammini* declined

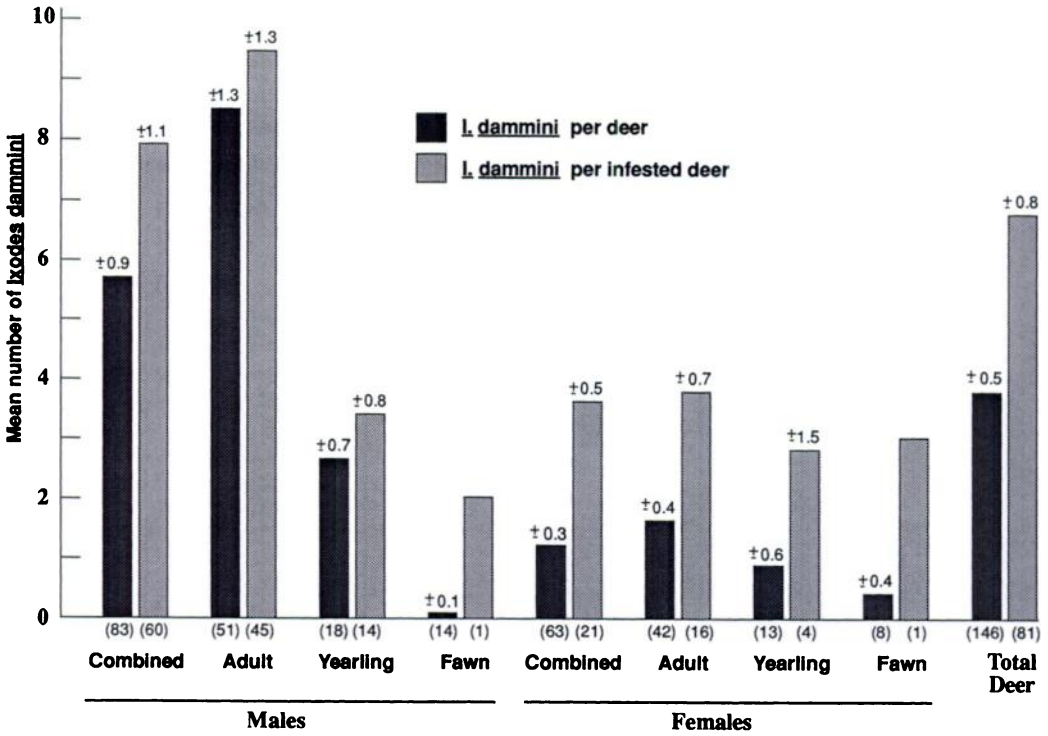

FIGURE 3. The mean number of *Ixodes dammini* found on one side of the neck of deer grouped by sex and age. Explanation of the dark and lighter bars and the numbers above and below the bars is given in Figure 1.

during the fall and early winter (Main et al., 1981).

Borrelia burgdorferi, whose identity was confirmed by species-specific monoclonal antibody analysis, was found in 22% of the *I. dammini*. Based on a Chi-square test (Rosner, 1990), no significant ($P > 0.05$) difference was seen in the prevalence of spirochetes between male and female ticks. *Dermacentor albipictus* was not analyzed for the presence of spirochetes. Previously, Magnarelli et al. (1986) found one tick with *Borrelia* spirochetes out of 157 ticks examined from 66 deer in Lyme disease endemic areas. Drew et al. (1988) found a 10% prevalence of *Borrelia* spirochetes in ticks in Minnesota, as determined by polyclonal antibody analysis of adult *I. dammini* collected during the fall of 1985 and 1986 from dogs after grouse hunting trips. Many of these infected ticks were collected in the east-central part of the state near the current study site. Based on polyclonal antibodies, prevalences of 35% and 43% were found in adult *I. dammini* (Callister et al., 1988, 1991) and 39% in nymphal populations (Godsey et al., 1987) in Wisconsin. By culturing of larval and nymphal midgut tissue, Anderson et al. (1987) demonstrated a prevalence rate of 75%. Using polyclonal antibodies 20% of 401 adult *I. dammini* removed from a single dog from Spooner, Wisconsin (USA) were posi-

TABLE 3. Total number of *Ixodes dammini* collected from one side of the neck of 146 white-tailed deer in Saint Croix State Park, Minnesota, November, 1989, and screened by indirect immunofluorescence (IFA) assay for *Borrelia burgdorferi*.

I. dammini	Number collected	Number tested by IFA	Number (percent) positive
Male	390	309	71 (23.0)
Female	159	126	26 (20.6)
Total	549	435	97 (22.3)

tive for *B. burgdorferi* (R. C. Johnson, unpubl.).

Approximately one of every four adult *I. dammini* in this study was infected with *B. burgdorferi* (Table 3), and although immature forms of *I. dammini* were not examined, a significant percentage of nymphal ticks probably was infected with the Lyme disease spirochete. We believe this to be true because most *B. burgdorferi* infections in *I. dammini* are acquired when larvae or nymphs feed on infected small mammals such as the white-footed mouse (*P. leucopus*), the primary reservoir of the spirochete in nature (Anderson et al., 1985). Transovarial transmission of the spirochete in *I. dammini* occurs at a rate of about 1% (Magnarelli et al., 1986; Piesman et al., 1986; Telford et al., 1988) and virtually no transmission occurs from white-tailed deer to feeding *I. dammini* (Telford et al., 1988). Therefore, we assume that the infected adult ticks in this study acquired the spirochete transstadially from nymphs or larvae infected while feeding on white-footed mice or other suitable hosts. We believe that the percentage of questing nymphs and adults infected with *B. burgdorferi* is sufficiently great that tick bites resulting from visiting this popular recreational area pose a significant risk to people and domestic animals. The public should be aware of appropriate measures to prevent tick bites, such as application of acaricide chemical sprays and thorough daily inspection of the body for ticks.

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