JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1988, p. 2632–2636
0095-1137/88/122632-05$02.00/0

Vol. 26, No. 12

Borrelia burgdorferi Infection Surrounding La Crosse, Wis.

STEVEN M. CALLISTER,[1,2] WILLIAM A. AGGER,[1,3]* RONALD F. SCHELL,[2,4] AND JAY L. E. ELLINGSON[1]

Microbiology Research Laboratory, Gundersen Medical Foundation,[1] and Infectious Disease Section, Gundersen Clinic,[3] La Crosse, Wisconsin 54601, and Wisconsin State Laboratory of Hygiene[4] and Department of Medical Microbiology,[2] University of Wisconsin, Madison, Wisconsin 53706

Received 18 April 1988/Accepted 9 September 1988

This investigation defined the extent of *Borrelia burgdorferi* infection surrounding La Crosse, Wis. White-footed mice, *Peromyscus leucopus* or *P. maniculatis*, were captured from sites in Wisconsin, Minnesota, and Iowa and cultured for *B. burgdorferi* to define the local boundaries of the midwestern Lyme disease area. All foci of *B. burgdorferi* infection (N1, N2, N3, and N4) were located north of interstate highway 90 except focus S2, which was south of the highway near Fort McCoy, Wis. The interstate highway may have been a barrier to deer movement which slowed the southward dispersal of *Ixodes dammini*. *B. burgdorferi* was isolated from 12 (63%) of the mice captured from site N4, which was adjacent to the western border of Fort McCoy. Unexpectedly, no *B. burgdorferi*-infected mice were isolated at site N0, located north of interstate highway 90 and enclosed by areas in which *B. burgdorferi* infection is endemic. This site is surrounded by natural barriers which may have slowed the spread of *I. dammini* by deer. The Wisconsin area in which *B. burgdorferi* is endemic should now include the surrounding area north of interstate highway 90 west from Fort McCoy to the Mississippi River. Additional studies are needed to define the rapidity, limits, and means of *I. dammini* dispersal into southern Wisconsin.

Lyme disease, named after the site of an epidemic of oligoarticular arthritis (31), is a multisystem infection often accompanied by a unique skin lesion, erythema migrans. Subsequent epidemiological studies have identified the deer tick, *Ixodes dammini*, as the primary vector of Lyme disease in North America (12) and a spirochete, *Borrelia burgdorferi*, as the causative agent of Lyme disease (6, 30). Lyme disease is now the most common tick-borne illness recognized in the United States (19).

This infection has been recognized in Europe since 1909 (1). In 1969, a grouse hunter from Taylor County, Wis., contracted the first reported case of erythema migrans with systemic illness in North America (27). Subsequently, additional cases of Lyme disease were reported from the northeastern United States (11, 31) and from northwestern Wisconsin and adjacent northeastern Minnesota (16, 17). Clinical and epidemiological studies now indicate that the range of *B. burgdorferi*-infected *I. dammini* may be increasing in Wisconsin (2, 13, 16, 18).

Lyme disease foci have been identified by clinical and serological studies of humans (11, 30, 31), the presence of *B. burgdorferi*-infected ticks (5, 6, 24), and serological studies of wild and domestic animals (23–25). Recently, the recovery of *B. burgdorferi* from white-footed mice, *Peromyscus leucopus*, has also proven useful for determining Lyme disease foci (4).

The purpose of this investigation was to examine white-footed mice captured from areas surrounding La Crosse, Wis., for *B. burgdorferi*, since this area is close to a previously recognized focus of *B. burgdorferi* infection at Fort McCoy, Wis. (2, 3, 18). Mice from other sites in Wisconsin, Minnesota, and Iowa were also cultured to define the local boundaries of *B. burgdorferi* infection.

MATERIALS AND METHODS

White-footed mice (*Peromyscus leucopus* or *P. maniculatis*) were captured from nine sites in Wisconsin at various intervals from July 1986 to September 1987 (Fig. 1). Samples were taken from sites north (N0, N1, N2, N3, N4) and south (S1, S2, S3, S4) of interstate highway 90 from Tomah, Wis., westward to La Crosse, Wis. In addition, sites near Winona, Minn. (M1), La Crescent, Minn. (M2), and Lansing, Iowa (I), were sampled. The sites were located in a nonglaciated area characterized by mixed hardwood-evergreen forests (15).

Mice were captured in Sherman box traps and similar homemade traps baited with peanuts. The traps were set along approximately straight-line transects. Eighty traps were set in groups of two at 75- to 100-ft (22.86- to 30.48-m) intervals. Transects were chosen to sample a variety of woodland habitats and were changed daily to avoid depletion of adult mice in an area. Traps were set on consecutive nights until approximately 20 mice were captured.

After being killed with chloroform, mice were identified as either *P. leucopus* or *P. maniculatus* (20). Ticks were removed from the ears of the mice with forceps, and their species and developmental stages were determined (26, 28).

Mice were then soaked with disinfectant (UKG; Dalco Inc., Minneapolis, Minn.), and their spleens and left kidneys were removed aseptically and cultured as previously described by Anderson et al. (4). Briefly, the tissues were forced through a 1-ml tuberculin syringe into Barbour-Stoenner-Kelly medium (4, 7) and vortexed for 1 min. Duplicate tubes of Barbour-Stoenner-Kelly medium (6 ml) containing 0.15% agarose (Seakem LE; FMC Corp., Marine Colloids Div., Rockland, Maine) were inoculated with 0.6 ml of each kidney or splenic suspension. Cultures were incubated in the dark at 31 to 33°C for 4 to 5 weeks and examined for spirochetes with dark-field microscopy.

Spirochetes were concentrated by centrifugation at 5,000 rpm (Sure Spin model 7040; Helena Laboratories) for 30 min and washed with phosphate-buffered saline (pH 7.2). After three washes, spirochetes were suspended and applied as a thin film on acid-cleaned slides. Slides were air dried overnight and fixed with acetone for 10 min. *B. burgdorferi* organisms were identified by using an indirect fluorescent

* Corresponding author.

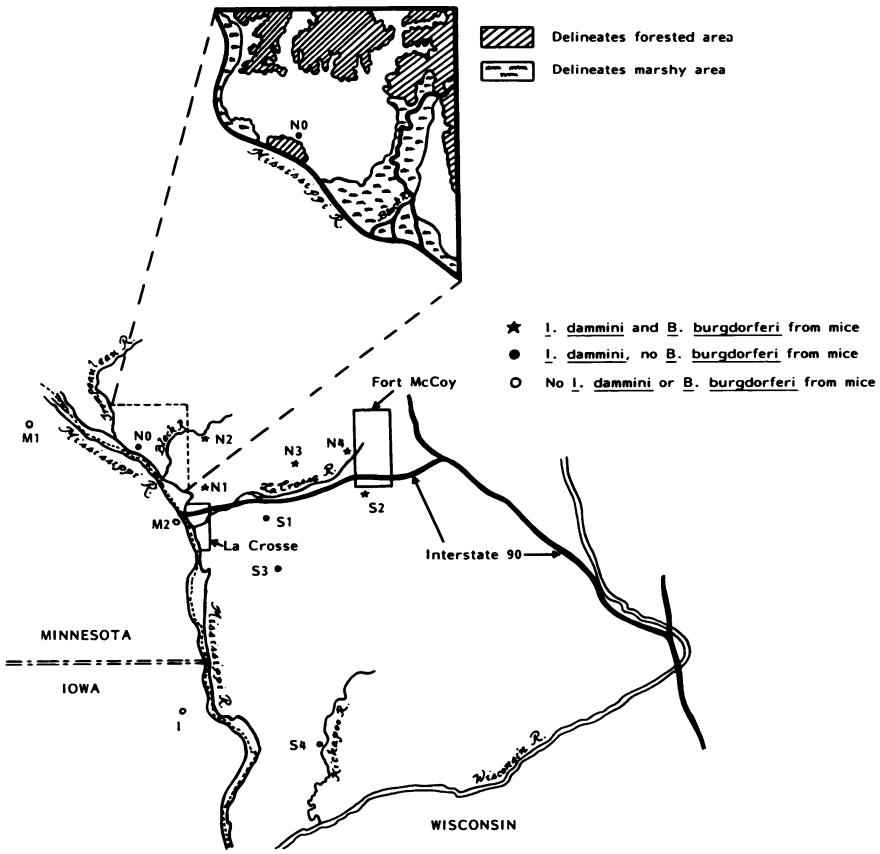

FIG. 1. Locations of sampling sites surrounding La Crosse, Wis.

antibody test with *B. burgdorferi*-specific monoclonal antibody H5332 (provided by Alan Barbour, University of Texas, San Antonio) which is reactive to a 31,000-molecular-weight surface protein (9).

Adult *I. dammini* ticks were collected by flagging the underbrush during October when ticks were questing. Flagging consisted of dragging a cardboard box covered with a white sheet through the underbrush and periodically examining the sheet for ticks. Sample sites were randomly flagged until 20 adult *I. dammini* ticks were captured. The tick midgut tissues were smeared onto a glass slide, fixed in acetone, and examined for spirochetes by direct fluorescent-antibody staining with a fluorescein-conjugated rabbit antibody (provided by Russell C. Johnson, University of Minnesota, Minneapolis) against *B. burgdorferi* B-31 (8).

RESULTS

One hundred thirty-two *P. leucopus* mice were captured at site N1 (Table 1). *B. burgdorferi* organisms were cultured from a total of 15 (11%) mice, with the highest seasonal infection rates being 25 and 30% during July 1986 and June 1987, respectively. Only one *P. leucopus* was infected with *B. burgdorferi* in February and March 1987. No spirochetes were recovered from mice during October and December 1986 and April 1987.

Mice were parasitized by immature *I. dammini* during July 1986 and April, June, August, and September 1987 (Table 1). Larvae were most abundant during July 1986 and June 1987. A mean of less than one larva per mouse was recovered from mice during April, August, and September 1987.

At the remaining sites (N0, N2, N3, N4, S1, S2, S3, S4, M1, M2, and I), 211 *P. leucopus* mice were captured (Table 2). *B. burgdorferi* organisms were isolated from 26 (12%) of these mice. However, only foci N2, N3, N4, and S2 yielded infected mice. Eighteen *P. maniculatus* mice were also captured at sites N2 and N3. Three (17%) were infected with *B. burgdorferi*.

Both larval and nymphal *I. dammini* ticks were present on

TABLE 1. Prevalence of *P. leucopus* infected with *I. dammini* and *B. burgdorferi* at site N1

Yr and mo of collection	*B. burgdorferi*-infected mice/total mice (%)	Mean no. of *I. dammini* ticks per mouse[a]	
		Larvae	Nymphs
1986			
July	7/28 (25)	1.6	0
October	0/17	0	0
December	0/16	0	0
1987			
February	1/13 (8)	0	0
March	1/17 (6)	0	0
April	0/21	0.2	0.1
June	6/20 (30)	1.1	0.7
August-September	ND[b]	0.6	0.4
Total	15/132 (11)	0.3	0.2

[a] Only *I. dammini* ticks on ears were counted.
[b] ND, Not done.

TABLE 2. Prelavence of *Peromyscus* spp.[a] infected with *I. dammini* and *B. burgdorferi* at sites N0, N2, N3, N4, S1, S2, S3, S4, M1, M2, and I

Sample site (mo)	*B. burgdorferi*-infected mice/total mice (%)	Mean no. of *I. dammini* ticks per mouse[b]	
		Larvae	Nymphs
N0 (July)	0/25	0.2	0
N2 (June)	9/18 (50)	1.6	0.8
N3 (July)	7/24 (29)	0.6	0.04
N4 (July)	12/19 (63)	0.8	0.4
S1 (July)	0/16	0.4	0
S2 (September)	1/25 (4)	0.9	0.1
S3 (July)	0/18	0.3	0
S4 (July)	0/16	0.2	0
M1 (August)	0/21	0	0
M2 (July)	0/21	0	0
I (July)	0/26	0	0
Total	29/229 (13)	0.4	0.1

[a] Includes *P. leucopus* and *P. maniculatus*.
[b] Only *I. dammini* ticks on ears were counted.

mice from those sites in which *B. burgdorferi* was endemic. Only larval *I. dammini* ticks were recovered from the remaining Wisconsin sites. No *I. dammini* ticks were recovered from site M1, M2, or I.

The percentages of adult *I. dammini* ticks infected with *B. burgdorferi* from foci N2, N3, N4, and S2 were 55, 52, 30, and 35%, respectively (Table 3). Of the *I. dammini* ticks recovered from site S1, 50% were infected. However, only four ticks were examined. Adult *I. dammini* ticks were not found at sites N0, S3, and S4. Percentages of infected females and males were similar.

DISCUSSION

Previous investigations have shown that Fort McCoy, Wis., is a focus for *B. burgdorferi* infection (2, 3, 18). Our study reveals that this area includes the terrain north of interstate highway 90 leading west to the Mississippi River. We believe this area is expanding. Outdoorsmen have reported increasing numbers of deer ticks in this area in recent years, and the number of human Lyme disease cases diagnosed at our institution has increased. In 1985, 27 human Lyme disease cases were diagnosed, while in 1987, 42 cases were diagnosed. Most human Lyme disease cases were contracted from areas north of interstate highway 90 in Wisconsin (unpublished data).

TABLE 3. Prevalence of adult *I. dammini* ticks infected with *B. burgdorferi* at sites N2, N3, N4, S1, and S2

Sample site	No. of ticks	No. (%) of *B. burgdorferi*-infected *I. dammini* ticks		
		Males	Females	Total
N2	22	6 (50)	6 (60)	12 (55)
N3	23	7 (54)	5 (50)	12 (52)
N4	20	3 (30)	3 (30)	6 (30)
S1	4	1 (50)	1 (50)	2 (50)
S2	26	4 (29)	5 (42)	9 (35)
Total	95	21 (41)	20 (45)	41 (43)

Anderson et al. (3) isolated *B. burgdorferi* at the highest frequency from mice captured from June through August. Similarly, we found that June and July yielded the highest percentage of infected mice. However, we also recovered *B. burgdorferi* from a few mice during February and March. This confirmed a previous observation (3) that a small percentage of mice stay infected over the winter and act as reservoirs for immature ticks which emerge in the spring.

Previous investigations in the northeastern United States showed that mice were heavily parasitized by *I. dammini* nymphs from May through June (26, 29) and by *I. dammini* larvae from July through September (6, 10, 21). Our results confirmed these findings. We found, however, that larval and nymphal *I. dammini* densities were lower than levels reported from other Wisconsin areas (2, 18). We counted only ticks from the ears, while other investigators counted *I. dammini* ticks on all parts of the mice (2, 18). In addition, annual weather variations may have also affected tick and mouse populations, since we trapped mice during different years.

I. dammini infestations of 32 larvae per mouse have been observed in the northeastern U.S. (21), which is considerably higher than the numbers of immature ticks found on mice in Wisconsin. These differences may be due to the composition and population densities of *I. dammini* hosts (18). Higher concentrations of *I. dammini* ticks probably exist in Wisconsin, especially in the northern portions, where investigators have found larger numbers of adult *I. dammini* ticks on deer (16). We did not investigate tick densities on other small mammals. Since our data were collected, chipmunks have been shown to be important hosts for *I. dammini* in Wisconsin (18).

All foci of *B. burgdorferi* infection except focus S2 (N1, N2, N3, and N4) were located north of interstate highway 90. The prevalence of *B. burgdorferi* was highest (63% positive) in *P. leucopus* mice captured at focus N4, which was adjacent to the western border of Fort McCoy, Wis. While these results are lower than those previously reported from Fort McCoy (2), they confirm that *B. burgdorferi* infection is prevalent in that area.

Interestingly, only immature *I. dammini* ticks and no *B. burgdorferi*-infected mice were isolated at site N0, indicating that this site is not yet a focus for Lyme disease (Fig. 1). These results were unexpected, since N0 is north of the interstate and is enclosed by an area in which Lyme disease is endemic. However, the site is surrounded by low, marshy areas of the Trempeauleau and Black Rivers, which often flood during the spring and fall. These flooded areas may hinder tick dispersal, since ticks and eggs may be destroyed during biannual high water. Additionally, treeless farming areas insulate site N0 for several miles in all directions. This may cause minimal deer traffic from surrounding areas in which Lyme disease is endemic, since large open fields must be traversed.

The interstate highway may have acted as a barrier to deer traffic and temporarily slowed the southern dispersal of *I. dammini*. However, *I. dammini* ticks were abundant south of the interstate at Fort McCoy (S2), and 35% of adult *I. dammini* ticks at focus S2 were infected with *B. burgdorferi*. In our study, only one (4%) mouse harbored *B. burgdorferi* at focus S2. This may be an inaccurate representation of the prevalence of *B. burgdorferi* in that area, since this site was sampled in September, when percentages of infected mice were declining. Adjacent areas, as well as the median, are

forested along interstate highway 90 in Fort McCoy, which probably increases the crossing frequency of deer carrying infected ticks.

Only four adult *I. dammini* ticks were captured at site S1 during 8 h of flagging on days when ticks had been successfully captured from other foci. Two (50%) of the adult ticks harbored *B. burgdorferi*. This site seems to be on the southern edge of deer tick dispersal, since no immature *I. dammini* ticks were present on *P. leucopus* during the previous summer (unpublished data).

These findings support previous investigations indicating that *B. burgdorferi*-infected *I. dammini* ticks are dispersing in a southward direction (16, 18). The larger deer populations in southern Wisconsin (18) may accelerate the dispersal rate of infected ticks. Unless the Wisconsin River can act as an effective barrier, there may be a rapid spread of *B. burgdorferi* further into southwestern Wisconsin and northwestern Illinois. These areas have abundant hardwood forests, which are excellent habitats for deer and ticks.

Small numbers of immature *I. dammini* ticks and no *B. burgdorferi*-infected mice were present at sites S3 and S4 in southern Wisconsin. *I. dammini* infestation in these areas is probably not due to dispersal by deer. Rather, *I. dammini* may have been introduced by other methods, such as transmission by biting flies (22), carriage by passerine birds (5, 26), transport on hunting dogs (18), or conveyance of tick-infected deer carcasses during hunting season (18). Sites I, M1, and M2 were also not yet infected with *I. dammini*. The Mississippi River may have prevented dispersal of tick-infected mice and deer.

Foci of Lyme disease are defined frequently by the presence of *B. burgdorferi* in ticks (5, 6, 24). In this study, examining captured *I. dammini* midguts for *B. burgdorferi* was effective only when investigating an area in which high levels of *B. burgdorferi* infection were present. Approximately 50% of adult *I. dammini* ticks from all foci were infected with *B. burgdorferi*, further confirming their high frequency of infection. However, capturing sufficient numbers of ticks was difficult unless ticks were questing.

Our results confirm that culturing *B. burgdorferi* from white-footed mice is valuable for mapping areas in which Lyme disease is endemic. In this study, *I. dammini* would not have been identified at sites N0, S3, and S4 if mice had not been examined. We also recovered *I. dammini* and *B. burgdorferi* from *P. maniculatus*, a species closely related to *P. leucopus* which has been experimentally shown to harbor *B. burgdorferi* (14). La Crosse, Wis., is on the southern edge of a large area, which encompasses northwestern Wisconsin and northeastern Minnesota, in which Lyme disease is endemic. We believe that this area is expanding, and additional studies are needed to determine the rapidity, limits, and means of *I. dammini* dispersal into southern Wisconsin.

ACKNOWLEDGMENTS

We thank Kay Case and Karen Brand for expert technical assistance and help with collecting specimens. Gerry Lower provided valuable assistance with the collection and transport of mice.

This research was supported by the Gundersen Medical Foundation, Ltd., La Crosse, Wis.

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