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Survey for *Ixodes* spp. and *Borrelia burgdorferi* in Southeastern Wisconsin and Northeastern Illinois

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Forested areas adjacent to Milwaukee, Wis., and Chicago, Ill., were investigated for rodents and ticks infected with *Borrelia burgdorferi*, the causative agent of Lyme disease. White-footed mice (*Peromyscus leucopus* or *Peromyscus maniculatus*), meadow voles (*Microtus pennsylvanicus*), and eastern chipmunks (*Tamias striatus*) were captured; and specimens from these animals were cultured for *B. burgdorferi* to define whether the midwestern Lyme disease area currently encompasses these large metropolitan centers. During 1988, *B. burgdorferi* was successfully cultured from the tissues of two *M. pennyslvanicus* voles captured from the Chicago area. However, no *Ixodes* spp. ticks were captured. None of 274 animals captured from sites I3 and 12 additional sites in Wisconsin and Illinois during the summer of 1989 were infected with *B. burgdorferi* or *Ixodes* spp. In addition, no ticks were recovered when the underbrush in 11 contiguous areas was flagged. Apparently, *B. burgdorferi* is rarely found in these areas because of the absence of the appropriate tick vectors. Further studies are needed to monitor the dispersal of *B. burgdorferi*-infected *Ixodes dammini* into this heavily populated midwestern region.

Lyme disease is a multisystem disorder affecting the skin, joints, nervous system, and heart (19, 20, 22). This disease occurs in humans (21) and domestic and wild animals (5, 17) after infection with the spirochete *Borrelia burgdorferi* (10, 21). Its major vectors are ticks of the *Ixodes ricinus* complex (7, 10), which includes *Ixodes dammini* and which is endemic in the eastern and midwestern United States (5, 12), and *Ixodes pacificus*, which is endemic in the western United States (9). At this time, Lyme disease is the most common tick-associated illness in the United States (14). If current trends continue, this disease may soon become the most recognized tick-associated illness in the world.

Small mammals, such as the white-footed mouse (*Peromyscus leucopus* and *Peromyscus maniculatus*) and the eastern chipmunk (*Tamias striatus*), are important *B. burgdorferi* reservoirs and hosts of immature *I. dammini* (2, 5, 11). The isolation of *B. burgdorferi* from the tissues of these rodents is valuable for identifying Lyme disease foci (2, 12). In addition, endemic foci have been identified by flagging the underbrush for adult *I. dammini* and examining the captured ticks for *B. burgdorferi* (4). We used these methods to determine whether forested areas adjacent to Milwaukee, Wis., and Chicago, Ill., were endemic for *Ixodes* spp. ticks and *B. burgdorferi*. These metropolitan areas were investigated since they have populations of white-tailed deer (*Odocoileus virginianus*) in contiguous forested areas biologically similar to the expanding midwestern Lyme disease area located in northeastern Minnesota and northwestern Wisconsin (12, 15). In addition, increasing numbers of patients from these areas are being diagnosed as having Lyme disease. Our results clarify the extent of *B. burgdorferi* infection in the upper Midwest.

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Study sites I1 to I7 and W1 to W8 were located in forest conservation district parks, which ranged in size from approximately 500 to 4,000 acres (200 to 1,600 ha), throughout northeastern Illinois and southeastern Wisconsin, respectively (Fig. 1). All of the study sites consisted of a habitat characterized by mature, deciduous oak-hickory forests interspersed with prairie grasslands and containing populations of white-tailed deer (*O. virginianus*).

White-footed mice (*P. leucopus* and *P. maniculatus*), meadow voles (*Microtus pennsylvanicus*), and eastern chipmunks (*T. striatus*) were captured unharmed in homemade traps baited with peanuts as described previously (12). Briefly, the traps were set in approximately straight-line transects. A total of 75 to 150 traps were set in groups of two at 75- to 100-ft (22.86- to 30.48-m) intervals along transects chosen to sample a variety of habitats. The trapping transects were changed daily, and traps were set in sample areas on successive days until approximately 20 animals were captured. Rodents were sacrificed by using CO_2 and visually examined for ticks, which were removed and identified to the species level.

After examination, the rodents were soaked in disinfectant (UKG; Dalco Inc., Minneapolis, Minn.). Spleens, kidneys, and urinary bladders were aseptically removed and cultured as described previously (11, 12). Dissected tissues were forced through 1-ml tuberculin syringes into modified Barbour-Stoenner-Kelly medium (6, 13) and vortexed at high speed for 1 min. Duplicate tubes containing 5.4 ml of modified Barbour-Stoenner-Kelly medium with an additional 0.15% agarose (Seakem LE; FMC Corp., Marine Colloids Div., Rockland, Maine) were inoculated with 0.6 ml of each splenic, kidney, or bladder suspension. All cultures were incubated in the dark at 32°C for 3 weeks and examined for spirochetes by dark-field microscopy.

Flagging for ticks consisted of dragging flattened card-

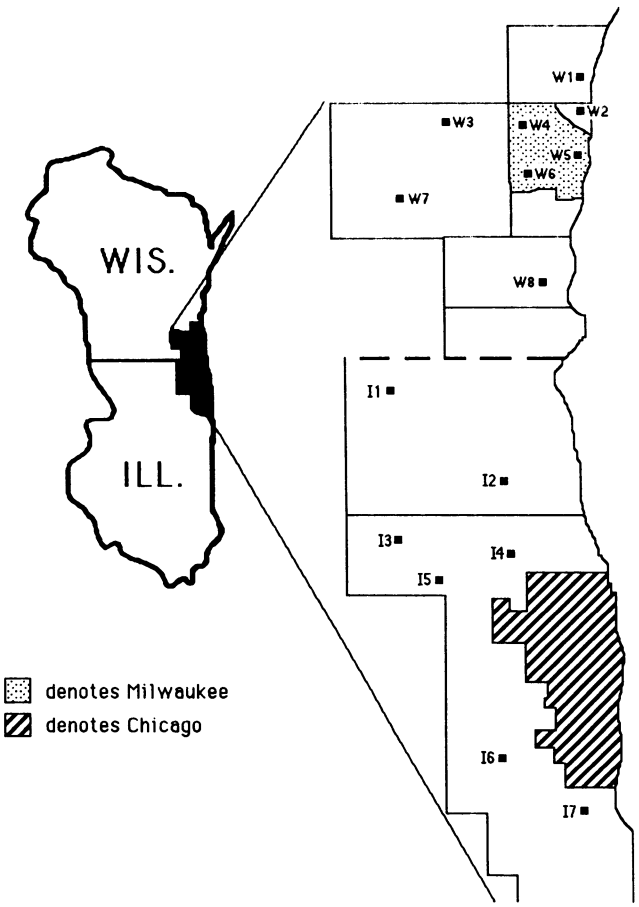

FIG. 1. Locations of sampling sites surrounding Milwaukee, Wis., and Chicago, Ill.

board boxes covered with white cotton sheets or burlap cloth through the underbrush and periodically examining the cloth for ticks. Sample sites were randomly flagged for 5- to 8-h intervals during the spring and fall when adult ticks were questing in Wisconsin.

All captured ticks were examined for *B. burgdorferi* infection by indirect fluorescent-antibody staining (7). Midgut tissues were smeared onto a glass slide, air dried, and fixed in acetone. After fixation, the slide was stained with a polyvalent rabbit antibody reactive against *B. burgdorferi* B-31 (Russell C. Johnson, University of Minnesota, Minneapolis), overlaid with fluorescein-conjugated anti-rabbit antibody, and examined with a fluorescence microscope.

Spirochetes from tissue cultures were identified by using an indirect fluorescent-antibody test (8) with *B. burgdorferi* genus- and species-specific monoclonal antibodies H9724 (Symbicom, Umea, Sweden) and H5332 (Alan Barbour, University of Texas, San Antonio), which are reactive to 41,000- and 31,000-molecular-weight surface proteins, respectively.

During an initial survey in 1988, a total of 23 mice (which included *P. leucopus* and *P. maniculatus*) and three meadow voles (*M. pennsylvanicus*) were captured from sites I3, I4, and I5. No rodents were parasitized by ticks. However, three adult wood ticks (*Dermacentor variabilis*), none of which were infected with *B. burgdorferi*, were collected from the outer clothing of field workers.

TABLE 1. Prevalence of *Peromyscus* spp.,[a] *M. pennsylvanicus*, and *T. striatus* infected with *B. burgdorferi* at sites I1 to I3, I6, I7, and W1 to W8 during May through August 1989

Sample site	No. *B. burgdorferi* infected/total		
	Mice	Voles	Chipmunks
I1	0/3	NC[b]	NC
I2	0/20	NC	NC
I3	0/19[c]	0/1	NC
I6	0/7[d]	NC	NC
I7	0/3	NC	NC
W1	0/15	0/4	0/3
W2	0/24	NC	0/12
W3	0/14	NC	0/1
W4	0/18	0/1	NC
W5	0/17	0/4	0/2
W6	0/29	NC	0/12
W7	0/27	0/1	0/4
W8	0/26	0/7	NC
Total	0/222	0/18	0/34

[a] Includes *P. leucopus* and *P. maniculatus*.
[b] NC, None captured.
[c] One *D. variabilis* larva and one *D. variabilis* nymph were recovered from a *P. leucopus* mouse.
[d] A total of six *D. variabilis* larvae were recovered from five *P. leucopus* mice.

Cultures of tissue from the 23 mice were negative for *B. burgdorferi*. However, spirochete isolates were recovered from the kidneys, spleens, and bladders of two (67%) of the *M. pennsylvanicus* voles captured from site I3. These spirochetes were identified as *B. burgdorferi* after indirect fluorescent-antibody staining by using *Borrelia* spp.-specific monoclonal antibody H9724 and *B. burgdorferi*-specific monoclonal antibody H5332.

In an attempt to capture adult *I. dammini*, site I3 was flagged for ticks for three 5- to 8-h intervals on different days during October 1988 when adult ticks were questing in Wisconsin. During this time, 68 adult *I. dammini* were recovered from an endemic site in Wisconsin. Indirect fluorescent-antibody examinations of the midguts of these ticks revealed that 24 (35%) were infected with *B. burgdorferi*. In contrast, no ticks of any kind were recovered from site I3.

Because *B. burgdorferi* was successfully isolated from site I3, we expanded our efforts during the spring and summer of 1989 to include sites southward around Chicago and northward into areas surrounding Milwaukee. During May through August, when small animals in the Midwest are most often parasitized by *I. dammini* ticks and infected with *B. burgdorferi* (1, 12), rodents were captured from four and eight additional sites in northeastern Illinois and southeastern Wisconsin, respectively (Fig. 1). In addition, 19 *Peromyscus* mice and 1 *M. pennsylvanicus* vole were captured from site I3 in an attempt to reisolate *B. burgdorferi* from this previously positive site.

Five of the white-footed mice captured from site I6 were parasitized by a total of six *D. variabilis* larvae (Table 1). One *D. variabilis* larva and one *D. variabilis* nymph were recovered from a *P. leucopus* mouse captured from site I3. None of the recovered immature *D. variabilis* ticks were infected with *B. burgdorferi*. The number of animals sampled from sites I1, I6, and I7 was low because of the difficulty in capturing seemingly scarce animals. The data were in-

cluded since they help to demonstrate the scarcity of ticks in this region of the United States.

No ticks were present on animals captured from southeastern Wisconsin, and *B. burgdorferi* was not isolated from these Wisconsin or Illinois sites during the summer of 1989. In contrast, 34 *P. leucopus* mice were captured from the Wisconsin endemic focus during May through August 1989. Thirty-six *I. dammini* nymphs and 78 *I. dammini* larvae were removed from these animals. Examinations of the midguts of these ticks revealed that 8 nymphs (22%) and 10 larvae (13%) were infected with *B. burgdorferi*. In addition, *B. burgdorferi* isolates were recovered from the tissues of 13 (35%) of the *P. leucopus* mice captured from the Wisconsin Lyme disease focus.

Further attempts to capture any ticks by flagging 11 additional sites in northeastern Illinois for one or two 5- to 8-h intervals during October 1989, when 80 adult *I. dammini* ticks (38 [48%] positive for *B. burgdorferi*) were captured by flagging in the Wisconsin endemic focus, were unsuccessful. In addition, flagging intervals were repeated at these sites during June 1990, but no ticks were recovered.

Previous investigations have established the fact that there is an endemic midwestern Lyme disease area in northeastern Minnesota and contiguous northwestern Wisconsin (12, 15) and that this area is expanding (12, 16). In this study, *B. burgdorferi*-infected *I. dammini* ticks and rodents were easily captured from a site within this Lyme disease area. In contrast, *I. dammini* ticks could not be captured from southeastern Wisconsin or northeastern Illinois. In addition, *B. burgdorferi* was rarely recovered from rodents from only one northeastern Illinois site. These results confirm that *I. dammini* ticks and *B. burgdorferi* are not established in northeastern Illinois and southeastern Wisconsin and that these areas are not yet part of the midwestern Lyme disease area. Because of this, it is unlikely that Lyme disease is commonly acquired locally in the immediate Milwaukee and Chicago vicinities.

There are several possible explanations why *B. burgdorferi* was isolated from site I3 during 1988 and was absent during 1989. Because no *I. dammini* ticks were captured, it is possible that a previously undefined *B. burgdorferi* transmission vector exists. For instance, biting flies, such as deerflies and horseflies, and mosquitoes have been shown to be infected with *B. burgdorferi* (18). However, it has not been demonstrated that these insects can successfully transmit *B. burgdorferi*, and these flies do not routinely feed on rodents.

Other possible vectors include fleas, mites, and lice. They have not been shown, however, to be efficient vectors. We examined all of the internal organs of 23 fleas (*Orchopeas leucopus*) removed from 15 of the *P. leucopus* mice captured from the site in the Wisconsin endemic Lyme focus. The mice were either infected with *B. burgdorferi* or being parasitized by *B. burgdorferi*-infected immature *I. dammini*, and none of the internal organs of the fleas were infected with *B. burgdorferi*. Until further studies clearly establish other possible *B. burgdorferi* vectors, *I. dammini* is the main Lyme disease vector in the upper Midwest.

There are multiple ways that *B. burgdorferi*-infected *I. dammini* could be periodically introduced into this large metropolitan area. For instance, the I3 site was located in a bird sanctuary, and larval and nymphal *I. dammini* have been shown to feed on passerine birds (3). Therefore, *B. burgdorferi*-infected immature *I. dammini* ticks could be introduced to this site via migratory birds. Small rodents could be infected by these bird-carried ticks and remain infected with *B. burgdorferi* into the following summer (1, 12). If the mature ticks were then unable to complete their life cycles because of low numbers of other adults or climatic factors, *I. dammini* infestation would not persist. Introductions of *I. dammini* could also occur via transport on humans or animals, such as dogs, recently parasitized in other endemic foci.

Our results confirm that the metropolitan areas of southeastern Wisconsin adjacent to Milwaukee and northeastern Illinois around Chicago are not currently endemic for Lyme disease. However, we successfully isolated *B. burgdorferi*, and these areas have large populations of mice and deer, which are essential for the survival of *I. dammini* ticks. Future epidemiological studies are needed to monitor for the establishment of *B. burgdorferi* and its vector, *I. dammini*, into this heavily populated midwestern area.

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