Identification of Endemic Foci of Lyme Disease: Isolation of Borrelia burgdorferi from Feral Rodents and Ticks (Dermacentor variabilis)

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Borrelia burgdorferi, the etiological agent of Lyme disease, was isolated from the blood, kidney, spleens, eyes, or livers of white-footed mice (Peromyscus leucopus) (n = 19 of 22) and from the blood, kidney, or spleens of eastern chipmunks (Tamias striatus) (n = 2 of 2) captured at three foci for Lyme disease in eastern Connecticut. These bacteria were cultured most frequently from spleens (n = 19) and kidneys (n = 15). B. burgdorferi persisted in one mouse for at least 60 days. One spirocheticemic mouse had infected Ixodes dammini and Dermacentor variabilis larvae attached, suggesting that these ticks may have acquired spirochetes from the host. Spirochetes isolated from P. leucopus, T. striatus, and D. variabilis larvae were serologically and genetically indistinguishable from reference B. burgdorferi isolates. We conclude that isolation of spirochetes from feral rodents is a method for identifying endemic areas of Lyme disease.

Borrelia burgdorferi, a newly described spirochete (14) that is transmitted by Ixodes dammini in the northeastern United States, is the etiological agent of Lyme disease (9). Numerous foci of this disease have been identified by clinical and serological studies of humans (8, 18, 22, 24), by determining the prevalence of ticks infected with B. burgdorferi (1–3, 9, 17, 22), and by serological studies of wild and domestic animals (16–19). Although this spirochete has been isolated from human blood, skin, and spinal fluid (6, 22) and from the blood of wild and domestic mammals (1–3, 7, 15; K. I. Loken, C. Wu, R. C. Johnson, and R. F. Bey, Proc. Exp. Biol. Med., in press), relatively few of these isolations have been subcultured. The frequent recovery of this bacterium from spleens and kidneys of experimentally infected hamsters (13) led us to attempt isolations from various organs of wild rodents. We report a relatively high recovery rate of B. burgdorferi from white-footed mice (Peromyscus leucopus) captured in three Connecticut areas and propose that this is a method for identifying foci of Lyme disease and for determining the geographic distribution of the spirochete. Additionally, we report the first isolations of B. burgdorferi from eastern chipmunks (Tamias striatus) and Dermacentor variabilis larvae.

MATERIALS AND METHODS

Rodents were captured alive in Sherman box traps from July through September in East Haddam, East Lyme, and Waterford, Conn. Lyme disease has been diagnosed in humans living in these communities. All locations are infested with I. dammini and consist of a mixture of hardwood forests, grasslands, and shrubs.

The tissues of 22 P. leucopus and 2 T. striatus were cultured in Barbour-Stoenner-Kelly (BSK) medium with or without 0.15% agarose (Seakem LE; FMC Corp., Rockland, Maine) (4, 13). With one exception, all animals were dissected within 72 h after capture (one mouse was sacrificed 60 days postcapture). Two drops of heparinized or untreated blood drawn from the heart was inoculated into 8 ml of medium. Whole kidneys, spleens, eyes, and liver lobes were triturated under aseptic conditions in 2 ml of medium, and 0.1-ml portions were inoculated into 8 ml of medium. Cultures were maintained at 34°C for 4 to 6 weeks and examined for spirochetes by dark-field microscopy. Organs from 13 mammals triturated in 2 ml of BSK medium without agarose were placed in 8-ml polystyrene screw-capped tubes and shipped in insulated cartons containing frozen cold packs by overnight courier to the University of Minnesota where duplicate 1:10 dilutions of the triturates were cultured in BSK medium at 30°C.

Ticks removed from two P. leucopus were identified, and their midgut tissues were examined for spirochetes by direct immunofluorescence with fluorescein isothiocyanate-labeled rabbit antibody against B. burgdorferi (provided by Allen C. Steere, Yale University, New Haven, Conn.) and by culture in BSK medium.

The identity of spirochetes recovered from rodents and D. variabilis larvae was made by reacting Swiss mouse antiserum prepared to B. burgdorferi isolate CT 2591 in indirect immunofluorescence tests (3). Monoclonal antibody H5332, reactive with the 31,000-molecular-weight surface protein of B. burgdorferi, was provided by Allan Barbour, Rocky Mountain Laboratories, Hamilton, Mont., and was used to confirm the identity of these isolates (5). Here whole spirochetes isolated from each infected rodent were washed and concentrated in phosphate-buffered saline (pH 7.2), mixed with a suspension of washed guinea pig erythrocytes, and applied as a thin film on glass slides. Preparations were allowed to dry for 2 h before fixation in acetone for 10 min. The indirect fluorescent antibody procedures have been described (5, 9). Additionally, spirochetes cultured from one T. striatus, one P. leucopus, and one D. variabilis larva were characterized genetically by DNA homology studies by using the DNA filter method (12, 14).

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RESULTS

All rodents (2 T. striatus and 7 P. leucopus) collected from East Lyme, 9 of 11 P. leucopus taken from East Haddam, and 3 of 4 P. leucopus captured from Waterford harbored spirochetes (Table 1). B. burgdorferi organisms were isolated from spleens, kidneys, eyes, blood, or livers. An infected kidney was obtained from one P. leucopus examined 60 days post-capture. Spirochetes grew well in all primary cultures and subsequent subcultures.

Two or more tissues from the same host frequently yielded spirochetes. For example, B. burgdorferi organisms were isolated from five tissue samples (blood, liver, spleen, kidney, and eye) of one P. leucopus and from two tissue samples of both T. striatus. Spirochetes were cultured most frequently from spleens (n = 19) and kidneys (n = 15).

Comparative studies on isolation procedures conducted at two distant laboratories showed that many positive cultures were obtained in both Connecticut (n = 43) and Minnesota (n = 30) (Table 1). However, there was a higher yield at the latter location (e.g., 12 of 13 spleens and 9 of 9 left kidneys were positive), where triturated tissues were inoculated into duplicate tubes of medium kept at 30°C.

Spirochetes were detected by direct immunofluorescence of midgut tissues from three of three larval and eight of nine nymphal I. dammini and were isolated from one of two larval D. variabilis removed from a P. leucopus with an infected kidney. Two other D. variabilis larvae collected from another mouse (organs not cultured), which died after capture, also contained spirochetes.

The spirochetes isolated from D. variabilis and tissues of P. leucopus and T. striatus were serologically indistinguishable from B. burgdorferi strains by indirect immunofluorescence. All isolates fluoresced at titers of ≥1:1.024 against Swiss mouse antisera to the previously identified CT 2591 isolate and also reacted with monoclonal antibody H5332 directed against the 31-kilodalton protein of B. burgdorferi, B31 (ATCC 35210). DNA hybridization studies with B. burgdorferi B31 DNA as the probe showed a percent relatedness of 80.5, 68, and 85% for isolates from a P. leucopus, a T. striatus, and a larva D. variabilis, respectively. These studies indicate the identity of all isolates as B. burgdorferi.

DISCUSSION

B. burgdorferi has been isolated from mammalian tissues, including humans, but recovery rates have been relatively low (1-3, 6, 7, 15, 22; Loken et al., in press). After repeatedly growing spirochetes from organs of experimentally infected Syrian hamsters, Johnson et al. (13) suggested that this bacterium might also be recovered from organs of wild mammals. The results of the present study confirm this hypothesis. Consistent isolation of B. burgdorferi from kidneys and spleens of P. leucopus and T. striatus shows this to be a suitable method for identifying areas where B. burgdorferi occurs in endemic proportions. Small mammals, such as P. leucopus, are preferred because they have extensive tick exposure (2, 21), they are easily caught throughout the year, and they have relatively small home ranges. Furthermore, since cultured spirochetes can now be accurately identified, the isolation of these bacteria from organs may be used to identify the species of mammals carrying B. burgdorferi and to determine the seasonal prevalence of infected hosts. Additionally, we have shown that triturated tissues are suitable for culture for at least 24 h. Thus, the initial processing of tissues could be performed under field conditions, and the samples could be cultured at a later time in an appropriately equipped laboratory.

The high percentage of infected rodents at the three Connecticut sites and the demonstration of spirochetes in 11 of 12 immature I. dammini feeding on one of the infected mice might explain, in part, the relatively high prevalence of infected I. dammini in Connecticut (2, 3, 17, 22). Clearly, many P. leucopus and T. striatus may be spirochetic and may serve as reservoirs in southeastern Connecticut.

Persistence of infection in wild rodents is unknown. Our recovery of spirochetes from a P. leucopus 2 months after capture and their isolation from a hamster 52 days postinoculation (13) demonstrate that B. burgdorferi survives in hosts for extended periods.

The isolation of B. burgdorferi from T. striatus brings the number of known infected mammalian species to eight; P. leucopus, Procyon lotor, (raccoon), Microtus pennsylvanicus, (meadow vole), Odocoileus virginianus (white-tailed deer), N. americanus, (woodland jumping mouse), humans, and domestic dogs (1-3, 6, 7, 15, 22; Loken et al., in press). Other mammals, such as D. variabilis (Virginia opossum) and S. carolinensis (gray squirrel), have been shown to have antibodies to this spirochete (16). Possibly most mammals parasitized by I. dammini will be found to harbor this bacterium.

Tick hosts of the I. ricinus complex are the chief vectors of B. burgdorferi (2, 3, 7, 9-11, 22, 23), though morphologically similar spirochetes have recently been detected in D. variabilis, H. leporispalustris, and A. americanum (2, 20). Isolates from D. variabilis larvae removed from P. leucopus in this study were both serologi-
cally and genetically indistinct from previously identified *B. burgdorferi* isolates. Although we do not know whether *D. variabilis* is an efficient vector of spirochetes, all motile stages of this tick feed on mammals and, along with *I. dammini*, may play a role in the ecology of Lyme disease.

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**LITERATURE CITED**


