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Isolation of the Lyme Disease Spirochete from Mammals in Minnesota (42100)

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Abstract. Lyme disease spirochetes were isolated from the kidneys of two *Peromyscus* spp. trapped in Minnesota in September and October 1983. No spirochetes were isolated from white-tailed deer (*Odocoileus virginianus*), red backed voles (*Clethrionomys gapperi*), or shrews (*Sorexy cinereus* and *Blarina brevicauda*). This is the first report of the isolation of the Lyme disease spirochete from the midwestern United States and isolations from these animals, which were free of ticks, suggest that the Lyme disease spirochete may persist in animal organs for months. © 1985 Society for Experimental Biology and Medicine.

Lyme disease, an infectious disease caused by a spirochete, was first recognized in 1975 (1). The illness usually begins with a skin lesion called erythema chronicum migrans (ECM) which may be followed weeks to months later by arthritic, neurologic, or cardiac sequellae (2, 3). The spirochete of Lyme disease is transmitted by ixodid ticks such as *Ixodes dammini* and *Ixodes pacificus* and was first isolated from *I. dammini* in 1982 (4). More recently, the spirochete was isolated from humans, white-footed mice, a white-tailed deer, a raccoon, and a dog in eastern United States (5–9).

Although Lyme disease was first recognized in Connecticut, cases have been reported from scattered areas of the United States [northeastern coast from Massachusetts to Maryland, Oregon, California (10), and Minnesota (11)].

We report efforts to isolate the spirochete from small mammals and white-tailed deer in Minnesota.

Materials and Methods. *Collection of animals.* Small mammals were sampled from two areas during the period from September 21 to October 26, 1983. The first sampling area was in eastern Pine County and consisted of a mixed hardwood forest, predominantly aspen, interspersed with small farms. This area is within the range of *I. dammini* and Lyme disease is present. The second area sampled was in northern St. Louis County, near the Canadian border. The area is characterized as a mixed conifer and hardwood forest. Lyme disease has not been diagnosed nor has *I. dammini* been observed in this area. Small mammals were collected with snap traps baited with peanut butter. Traps were visited three times daily; animals were collected, refrigerated, and transported to the laboratory for culture within 24–48 hr.

The head and neck of white-tailed deer were examined for the presence of *I. dammini* at a Department of Natural Resources check station in St. Croix Park, eastern Pine County, during a deer season conducted on November 5–6, 1983. Kidneys were collected from deer for bacteriological examination.

Isolation of spirochetes. An entire kidney from each small mammal or 1 to 3 g of kidney tissue from white-tailed deer was macerated and inoculated into 10 ml modified Kelly's medium (4). After allowing the larger tissue debris to settle, duplicate 1:10 dilutions of the culture supernatant were cultured. Kidneys were selected for culture because of convenience and adequate blood supply, and because they had been previously shown to be a good source of spirochete (12). Cultures were incubated at 30°C and samples of inoculated medium were examined by dark-field microscopy at 2-week intervals over a 6-week period. Positive cultures were frozen in 10% glycerol at −90°C.

Characterization of isolates. The identity of the isolated spirochetes as Lyme disease spirochetes was made with known antisera using the indirect immunofluorescent test (13) and by DNA homology studies (14).

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TABLE I. SPIROCHETE ISOLATIONS FROM KIDNEYS OF MINNESOTA MAMMALS

Collection site	Mammal	Number examined	Number positive
Pine County	*Sorex cinereus* (masked shrew)	31	0
	Blarina brevicauda (short-tailed shrew)	9	0
	Clethrionomys gapperi (southern red-backed vole)	15	0
	Peromyscus maniculatus[a] and *Peromyscus leucopus*[a] (woodland deer mouse, white-footed mouse)	16	2
	Odocoileus virginianus (white-tailed deer)	85	0
St. Louis County	*Sorex cinereus*	8	0
	Clethrionomys gapperi	44	0

[a] Combined due to the difficulty in distinguishing species in juveniles and subadults (15).

Results. *I. dammini,* a vector of the Lyme disease spirochetes, was present on 70% of the 85 white-tailed deer examined. However, none of the kidney cultures were positive for the Lyme disease spirochete (Table I). Cultural examination of the kidneys of 48 shrews and 59 voles also failed to detect the presence of this spirochete (Table I). In contrast, 12.5% of the 16 mice (*Peromyscus* spp.) examined harbored Lyme disease spirochetes as determined by dark-field examination kidney cultures (Table I, 21 days postinoculation). No *I. dammini* were observed on the mice.

Identity of the isolates was determined by the indirect immunofluorescent antibody test (IFA) and by DNA hybridization studies. Rabbit antisera to the original *I. dammini,* Shelter Island isolate (4), had a titration endpoint of 1:2048 with the homologous spirochete and with our isolates. In addition, both isolates reacted with monoclonal antibodies prepared to the 31K H5332 determinant of the Shelter Island spirochete (16). Using radiolabeled DNA from the *I. dammini,* Shelter Island spirochete, as a probe, a homology of approximately 66% was seen with the DNA of both mouse isolates. These results indicate that the Minnesota isolates are identical with the etiologic agent of Lyme disease (17). This represents the first isolation of the Lyme disease spirochete in the midwestern United States.

Discussion. Epidemiologic evidence suggests that Lyme disease is transmitted by *I. dammini* in Minnesota and Wisconsin (10). *I. dammini* is a three-host tick which may feed on birds and a variety of mammals (6). The immature stages of the tick are nonspecific in their choice of hosts while the adults are found in abundance only on white-tailed deer (18). Spirochetes have been found in nymphal and adult ticks, but not in unfed larval ticks, suggesting that larval ticks acquire the organism from animal reservoirs (7). Cases of Lyme disease in Minnesota are most prevalent in June and July (Minnesota Department of Health Disease Control Newsletter, Vol. II, March 1984). Nymphal ticks are the most prevalent stage of *I. dammini* during these months (18).

Spirochetes, which appear to be identical with the Lyme disease spirochete, were isolated from 2 of 16 *Peromyscus* spp. collected in an area within the range of *I. dammini.* Similar success in isolation of this spirochete from mice was reported from New York and Connecticut. Five of 77 *Peromyocus leucopus* trapped on Shelter Island, New York, had spirochetemia (5) while 1 of 79 *P. leucopus* from Connecticut yielded a positive culture (6).

The fact that no ticks were observed on the positive animals is probably related to the season in which the animals were collected. The two positive rodents were collected on October 26, 1983. Isolations from these animals which were free of ticks suggest that *Peromyscus* spp. may be important in the maintenance of infection over winter.

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