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## EXPERIMENTAL INFECTION OF THE EASTERN CHIPMUNK (*TAMIAS STRIATUS*) WITH THE LYME DISEASE SPIROCHETE (*BORRELIA BURGDORFERI*)

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**ABSTRACT:** Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is endemic in the northeast, north-central, and Pacific coastal states of the United States. The eastern chipmunk (*Tamias striatus*) is found throughout the disease-enzootic regions except along the Pacific coast, and may serve as an important reservoir host in some locations. To characterize their potential as a host, 11 adult chipmunks were inoculated with 10<sup>5</sup> spirochetes from strains of *B. burgdorferi* isolated from *Peromyscus leucopus* and *Ixodes scapularis* in a hyperendemic area of Westchester County, New York (USA). All inoculated chipmunks became infected. Spirochetemias were detected by isolating spirochetes in Barbour-Stoenner-Kelly media in eight of eight chipmunks, and lasted for 2 to 5 days. Spirochetes were isolated from the ears of all animals, starting at 1 wk and for  $\leq 4$  mo, and from various internal organs at 133 days post-inoculation. Laboratory-reared larval *I. scapularis* ticks became infected with spirochetes after feeding on two of the inoculated chipmunks.

**Key words:** *Borrelia burgdorferi*, Lyme disease, experimental infection, chipmunks, *Tamias striatus*, *Ixodes scapularis*, reservoir competence.

### INTRODUCTION

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is now the most frequently reported vector-borne human disease in the United States. The disease initially was recognized in two small foci, Connecticut and Wisconsin (USA), during the 1970's and now cases have been reported in 46 states, although the disease is indigenous in far fewer states (Schmid et al., 1985; Centers for Disease Control, 1991). Only 13 states had reports of more than 100 indigenous cases during 1990; eight of those states were in the northeast and the other five states were Georgia, Wisconsin, California, Michigan and Missouri (USA).

The principal hosts for *B. burgdorferi* are thought to be white-footed mice (*Peromyscus leucopus*) and deer mice (*P. maniculatus*) in the enzootic regions of the northeastern and midwestern states of the United States (Anderson and Magnarelli, 1984; Loken et al., 1985; Levine et al., 1985; Donahue et al., 1987; Godsey et al.,

1987). These mouse species also are major hosts for the immature stages of the primary tick vector, *Ixodes scapularis* (formerly *I. dammini*). The white-tailed deer (*Odocoileus virginianus*) is the major host for the adult stages of this tick. White-footed mice were the most important small mammal reservoir in coastal Massachusetts (USA) (Mather et al., 1989) and in Wisconsin (Godsey et al., 1987); however, the eastern chipmunk (*Tamias striatus*) was second to mice in prevalence of infection with *B. burgdorferi* and as a host of immature *I. scapularis* ticks. The eastern chipmunk is found in 12 of the 13 states that reported more than 100 human cases of Lyme disease in 1990 (Hall, 1981). This small mammal species could become an important host and reservoir in addition to, or in place of, white-footed mice or deer mice in some settings and locations such as residential yards in the northeast or woods and yards in the upper midwestern states, because of its local abundance and differences in habitat preferences and movement patterns.

Our objective was to evaluate the potential of the eastern chipmunk as a reservoir host for *B. burgdorferi*.

#### MATERIALS AND METHODS

Immature eastern chipmunks were captured in 1988 in Madison, Wisconsin, during a time when Madison was not known to be endemic for either *I. scapularis* ticks or indigenous human cases of Lyme disease (J. J. Kazmierczak, pers. comm.), and they were shipped to the Centers for Disease Control (CDC) laboratory in Fort Collins, Colorado (USA). They were maintained at the CDC laboratory for about 18 mo prior to the start of the experiment. The chipmunks were housed individually in clear plastic cages with wire tops and were provided with food and water *ad libitum* throughout the experiment except when they were exposed to ticks. Chipmunks were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Inc., Mundelein, Illinois, USA) for the collection of specimens and with 5 mg (25 to 30 mg/kg) of ketamine hydrochloride (Vetalar, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) for tick placement. Briefly, our experimental design was as follows. Eleven chipmunks in two separate experimental groups (Group I with three animals and Group II with eight animals) were inoculated with spirochetes and three control chipmunks were housed and handled in a similar fashion to the experimental ones. One of the control animals was sham inoculated with the culture media and sampled on the same schedule as the experimental animals in Group II and the other two controls were euthanized with carbon dioxide, and necropsied. The blood, ear, liver, spleen, kidney and bladder were tested after the termination of the experiment. All animals except for two controls were tested for current infection with spirochetes prior to their use in the experiment.

During the tick feeding phase of 6 days, two chipmunks from Group II (animal numbers 59 and 72) were individually held in wire cages suspended over water to collect detached, replete ticks. Approximately 50 *I. scapularis* larvae from a laboratory colony maintained at CDC were placed on each anesthetized chipmunk inoculated with spirochetes 4 mo previously. The engorged larvae collected from the water were placed in glass vials covered with fine nylon mesh and held in a desiccator jar with 70 to 80% relative humidity and maintained at about 24 C and 10 hr light per day. The ticks were allowed to molt to the nymphal stage before they were tested for spirochete infection. Twenty unfed larvae from the same batch of ticks

that had been applied to the chipmunks were tested for infection as well.

The isolates of spirochetes used to inoculate the chipmunks were originally isolated in Barbour-Stoenner-Kelly (BSK) culture medium (Barbour, 1984) from adult *I. scapularis* ticks collected within the Lyme disease hyperendemic region in Westchester County, New York (USA) (41°8'N, 73°48'W) during November 1989, and from ear biopsy tissue from a white-footed mouse (NY90-14) captured in Westchester County, New York in June 1990 (McLean, unpubl.). The isolates tentatively were identified by the direct fluorescent antibody (FA) test using a polyclonal antibody against *B. burgdorferi* produced locally in New Zealand white rabbits (Lord et al., 1992). The isolates were confirmed as *B. burgdorferi* by the indirect fluorescent antibody test using anti-*OpsA* monoclonal antibody H5322 (Barbour et al., 1983) with known strains of *B. burgdorferi* (B31) and *B. hermsii* as positive and negative controls, respectively. The isolates were passed in BSK medium and inocula of the second passage level containing about 10<sup>6</sup> spirochetes/ml of BSK medium were prepared. Three chipmunks in Group I each were inoculated subcutaneously (sc) with 0.1 ml of the tick isolate to determine the infectiousness of cultured spirochetes inoculated by needle and syringe. Following the conclusion of the preliminary experiment, eight chipmunks in experimental Group II each were inoculated subcutaneously with 0.1 ml or about 10<sup>5</sup> spirochetes of isolate NY90-14.

Blood specimens for culturing of spirochetes were collected weekly for the first 4 wk from the suborbital sinus with capillary pipets from chipmunks in Group I and daily for the first 8 days from chipmunks in Group II. Several drops of whole blood from each animal were dispensed directly into 7 ml of BSK medium for spirochete isolation. Skin biopsies from the ears were collected weekly for 8 wk. To collect ear biopsy specimens, the ear surfaces of anesthetized chipmunks were cleansed thoroughly with 70% ethyl alcohol (ETOH) before a small portion (3 to 4 mm in diameter) of ear tissue was punched out from each animal (Sinsky and Piesman, 1989). The tissue specimens were dipped first into 5% hydrogen peroxide solution and then 70% ETOH and washed with sterile phosphate buffered saline (PBS) before being placed into BSK medium for spirochete isolation.

Upon completion of the experiment, chipmunks were euthanized with carbon dioxide gas, and a blood sample was taken from the heart. Necropsies were performed on the eight chipmunks from Group II only, and the three control animals. During necropsy, small portions (about 1 cm<sup>2</sup>) of the liver, spleen, kidney, bladder and

TABLE 1. Isolation of spirochetes in BSK culture media from tissues of eastern chipmunks (*Tamias striatus*) inoculated with *Borrelia burgdorferi*.

Animal identification number	Blood										Ear tissue									
	Days postinoculation										Weeks postinoculation									
	0	1	2	3	4	5	6	7	8	0	1	2	3	4	5	6	7	8		
Control	—*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Experimental group II																				
52	—	+	+	+	+	+	—	—	—	—	+	—	+	+	+	+	+	+	—	—
58	—	—	+	+	+	—	—	—	—	—	+	+	—	+	+	+	+	+	+	+
59	—	+	—	+	+	+	—	—	—	—	—	+	+	+	—	+	+	+	+	+
61	—	+	—	+	—	—	—	—	—	—	+	+	+	—	+	+	+	+	+	+
67	—	+	+	+	+	—	—	—	—	—	+	+	+	—	+	+	+	+	+	+
68	—	—	+	+	+	—	—	—	—	—	+	—	+	+	+	+	+	+	+	+
71	—	—	+	+	+	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
72	—	—	—	+	+	+	—	—	—	—	+	+	+	+	+	—	—	—	+	+
Numbers positive	0	4	5	8	7	3	0	0	0	0	7	6	7	6	7	7	7	7	7	7

\* — = negative for isolation, + = spirochetes isolated in BSK medium.

ear were aseptically removed and cleansed as above, before maceration with a mortar and pestle in enough BSK medium to make approximately 10% suspensions. Nymphal ticks that had fed upon the two chipmunks from Group II were pooled in groups of five, their surface cleaned with hydrogen peroxide, alcohol, and PBS, and ground in BSK media with a mortar and pestle. The unfed larvae were pooled in two groups of 10 and treated similarly. The suspensions were centrifuged at 1,200 RPM for 10 min and 0.2 ml of each supernatant fluid was inoculated into 7 ml of BSK medium. All cultures were maintained at 34 C and examined weekly for 8 wk or until spirochetes were observed by dark-field microscopy. The spirochetes isolated in culture from the ticks feeding on the chipmunks were confirmed by the FA test. The data were analyzed with a chi-square test (Sokal and Rohlf, 1981).

## RESULTS

No spirochetes were isolated in culture media from blood or ear tissue taken from the chipmunks prior to the start of the experiment (Table 1). All 11 chipmunks inoculated with *B. burgdorferi* spirochetes became infected and the three control animals housed in the same room and from the same cohort remained free of infection (significantly different at  $P < 0.003$ ). Four weekly blood samples taken from the three chipmunks in Group I starting at 1 wk post-inoculation (PI) were negative for spi-

rochete isolation. However, spirochetes were isolated from the ear tissues of all three chipmunks first at 2 to 3 wk PI and for  $\leq 4$  mo. All eight of the chipmunks in Group II inoculated with the NY90-14 strain had a spirochetemia as detected by isolation in culture media. Spirochetemias were first detected on day 1 PI and lasted from 2 to 5 days with a mean ( $\pm$ SE) of  $3.4 \pm 0.3$  days (Table 1). All eight animals had a spirochetemia on day 3 PI. The ear punch tissue specimens were culture-positive for all eight animals in Group II (Table 1). Spirochetes were isolated from ear tissue in seven of eight animals by day 7 and from the remaining animal by day 14 PI. Ear tissues generally remained positive for all eight chipmunks for 8 wk PI, although isolates occasionally were not obtained from some chipmunks on some weeks (Table 1).

Larval ticks readily attached to the ears and head of the two exposed chipmunks, though one chipmunk was moderately successful in removing ticks. Ticks began detaching from chipmunks at 3 days and completed their feeding by day 6. Most of the engorged larvae molted to the nymphal stage. Spirochetes were isolated in culture and identified by the FA test from two of four tick pools from chipmunk 59

TABLE 2. Isolation of spirochetes in BSK culture media from tissues collected at necropsy from eastern chipmunks (*Tamias striatus*) 133 days after inoculation with *Borrelia burgdorferi*.

Animal identification number	Blood	Ear	Liver	Spleen	Kidney	Bladder
Controls (n = 3)	-	-	-	-	-	-
Experiment group II						
52	-	+	+	-	+	-
58	-	-	+	+	+	+
59	-	+	-	-	-	-
61	-	-	-	+	+	+
67	-	+	+	-	-	-
68	-	-	-	-	-	-
71	-	-	+	+	+	+
72	-	+	-	-	-	-
Number positive	0	4	4	3	4	3

- = negative for isolation, + = spirochetes isolated in BSK medium.

and two of five pools from chipmunk 72. Unfed larvae from the same batch of ticks were negative for spirochetes.

At necropsy on day 133 PI, no isolates were obtained from blood but isolates of *B. burgdorferi* were made from the liver, kidney, and ear tissue from four chipmunks, including both chipmunks used in the tick feeding experiment (Table 2). Isolates also were obtained from spleen and bladder tissue from three chipmunks. Isolates were made from all four internal organs from only two chipmunks and no isolates were obtained from any tissues at necropsy from one animal (No. 68) that previously was spirochetemic and had infected ear tissue on seven of the eight weeks PI (Tables 1, 2).

#### DISCUSSION

The eastern chipmunk was susceptible to experimental infection with two low-passage isolates of *B. burgdorferi* from New York and spirochetes could be easily recovered from various tissues from these animals. The uniform susceptibility, ease of isolating spirochetes, consistent spirochetemia of short duration and infection of ear tissue for up to at least 4 mo demonstrates that this species could be a good

reservoir host for the spirochete in nature. Field data supporting the natural infection of eastern chipmunks with *B. burgdorferi* have been obtained by spirochete isolation from chipmunks in southern Connecticut (Anderson et al., 1985) and by xenodiagnosis in Massachusetts where a 75% prevalence of infection was detected (Mather et al., 1989). Isolates have also been obtained in BSK media from eastern chipmunks captured in Westchester County, New York and St. Paul, Minnesota (USA) (McLean, unpubl.). Although we did not attempt to quantify the infectiousness of chipmunks for ticks, they were reservoir competent and infected colony-reared larval *I. scapularis* that fed upon them. The isolate and passage of *B. burgdorferi* inoculated and the route of infection by needle and syringe apparently did not prevent their competence to infect ticks in this experiment. Burgdorfer and Gage (1987) found no difference between the injection of tick tissues and the feeding of infected ticks in the response of cotton rats (*Sigmodon hispidus*) to infection with *B. burgdorferi*.

Based on the regular early appearance of spirochetemias, we believe chipmunks could be more infectious during the acute phase of their infection. The temporal pattern of spirochetemias detected by isolation in culture media was similar to spirochetemias detected in Syrian hamsters (*Mesocricetus auratus*). Hamsters had spirochetemias during the first 6 days of infection and blood cultures were negative for days 7 to 9 (Johnson et al., 1984). On the other hand, experimentally-infected cotton rats had consistent but intermittent spirochetemias for 3 to 4 wk and longer (Burgdorfer and Gage, 1987). Occasional isolations of spirochetes from blood have been obtained from naturally-infected insectivores (Telford et al., 1990), rodents (Anderson and Magnarelli, 1984; Anderson et al., 1987; Callister et al., 1989; Boyce et al., 1992), lagomorphs (Anderson et al., 1989) and carnivores (Anderson et al., 1983); from experimentally-infected wild

rodents (Burgess et al., 1986; Wright and Nielsen, 1990); and from a number of experimentally-infected laboratory animals such as hamsters and white mice (Moody et al., 1990), LEW/N rats (Barthold et al., 1988), and New Zealand white rabbits (Burgdorfer, 1984; Kornblatt et al., 1984). Some isolations from experimentally-infected animals were made weeks to months after inoculation; thus relapsing spirochetemias may occur (Burgdorfer and Gage, 1987). None of our animals were spirochetemic at necropsy.

It is very unlikely that these chipmunks were exposed to *B. burgdorferi* prior to their capture because of the lack of evidence for the tick vector or human Lyme disease cases in the area of their capture. Another factor is the short length of time of their potential natural exposure because they all were immature at the time of capture. In addition, blood and ear tissue from all chipmunks were found negative for infection with *B. burgdorferi* prior to their use in the experiment and all tissues tested from the three control animals were negative for infection at necropsy. Further evidence of the lack of prior exposure to the spirochete was the overwhelming acute infectious response for all 11 inoculated chipmunks which would not have occurred in immune animals. Serology would not have added much in disproving their prior exposure because the serologic tests are not always accurate, particularly for low-titered antibody, and the animals could have lost detectable antibody during the 2-yr interim period.

The eastern chipmunk is a common rodent in the deciduous forest regions of the eastern United States and prefers woods but also occurs in brushy areas (Snyder, 1982). It frequently occurs in the residential communities within the Lyme disease endemic regions of the northeast and north-central states, is common in and around residential yards (McLean, unpubl.) and has a relatively large home range of up to about 1 ha (Snyder, 1982). Therefore, this species has been shown to be a good res-

ervoir host for the Lyme disease spirochete and is a host for immature stages of the vector tick, *I. scapularis* (Mather et al., 1989), and it should be included in ecologic evaluations of the vertebrate hosts of Lyme disease and in host-targeted control methods applied in residential areas to control Lyme disease.

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