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ROLE OF THE EASTERN CHIPMUNK (*TAMIAS STRIATUS*) IN THE EPIZOOTIOLOGY OF LYME BORRELIOSIS IN NORTHWESTERN ILLINOIS, USA

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ABSTRACT: The role of the eastern chipmunk (*Tamias striatus*) in the epizootiology of Lyme borreliosis was evaluated in Castle Rock State Park, Illinois (USA), an enzootic region, from June to August 1993. Prevalence, intensity, and molting rate of immature *Ixodes scapularis* were determined for chipmunks, white footed mice (*Peromyscus leucopus*), and raccoons (*Procyon lotor*). Chipmunks were the primary host for *I. scapularis* nymphs and an important secondary host for *I. scapularis* larvae. Based upon ear punch biopsy analysis, *B. burgdorferi* prevalence in chipmunks was similar to that of mice in August and greater than that of mice in June and July. Thus we propose that chipmunks are the primary source of *B. burgdorferi* infection for *I. scapularis* nymphs and an important secondary source of infection for larvae.

Key words: *Tamias striatus*, *Peromyscus leucopus*, *Ixodes scapularis*, *Borrelia burgdorferi*, Lyme borreliosis.

INTRODUCTION

Lyme borreliosis is a tick-borne spirochetosis caused by *Borrelia burgdorferi* (Burgdorfer et al., 1982). Incidence of this disease has risen dramatically in the past decade, with approximately 10,000 new infections occurring each year (Centers for Disease Control, 1993). Lyme borreliosis is now the most prevalent vector borne disease in the United States. Yet control is impossible without a clear understanding of the ecology and enzootic cycle of Lyme disease in endemic regions. In this study, we clarify some of the relationships between etiologic agent, vector and reservoir species in a Lyme endemic region.

In the eastern United States, the vector for Lyme disease is the tick *Ixodes scapularis* (Kierans et al., 1996), also referred to as *Ixodes dammini* (Spielman et al., 1979). In the coastal northeastern United States, the primary wildlife reservoir for *B. burgdorferi* is the white footed mouse, *Peromyscus leucopus* (Levine et al., 1985). In that region, mice are major hosts for both immature stages of the vector (Levine et al., 1985), have a high incidence of spirochete infection (Magnarelli et al., 1984), and are highly infective to immature stages of the tick (Mather et al., 1990).

Borrelia burgdorferi was first identified in the midwestern United States in 1984 (Anderson et al., 1987) and in Illinois (USA) in 1987 (Nelson et al., 1991). Unlike the northeastern U.S., the distribution of *I. scapularis* infected with *B. burgdorferi* in the midwestern U.S. is very patchy with distinct enzootic foci (Kitron et al., 1992).

These multiple enzootic foci also differ ecologically from disease foci of the coastal northeastern U.S. In the midwestern U.S. mice are not the only significant hosts for immature *I. scapularis* in the region. In certain regions, the abundance of immature *I. scapularis* on eastern chipmunks (*Tamias striatus*) rivals tick densities on mice (Mannelli et al., 1993). Further, in laboratory studies, chipmunks can support spirochete infection and effectively transmit that infection to immature ticks (McLean et al., 1993). Thus Mannelli et al. (1993) hypothesized that chipmunks play a significant role in the epizootiology of Lyme disease in certain regions of the midwestern U.S.

Our objective was to further describe the role of chipmunks in the epizootiology of Lyme borreliosis by comparing the prevalence, intensity, and molting rates of *I. scapularis* on chipmunks, mice and raccoons (*Procyon lotor*), and determining

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the prevalence of *B. burgdorferi* in chipmunks and mice in Castle Rock State Park in northwestern Illinois.

MATERIALS AND METHODS

A 180 m × 300 m trapping grid of 60 traps set 30 m apart in a 6 × 10 pattern was established in a previously identified (Kitron et al., 1992) Illinois Lyme borreliosis focus at Castle Rock State Park (42°00′N, 89°20′w), in northwestern Illinois. The park is an 810-ha region on the banks of the Rock River which is comprised primarily of St. Peter's sandstone bluffs and ravines.

One 233 × 88 × 75 mm Sherman box trap (H. B. Sherman Trap Co., Tallahassee, Florida, USA) was placed at each of the 60 trap sites. Thirty Tomahawk live traps (model 102 chipmunk/rat: 41 × 13 × 13 cm) (Tomahawk Live Trap Co., Madison, Wisconsin, USA) were placed on the same grid because Sherman box traps are inadequate for sampling chipmunk populations (Yerger, 1953). A reduced trapping density was used for chipmunks as they generally have lower population density and larger home ranges than mice (Blair, 1942; Myton, 1974), and can therefore be adequately sampled with a smaller number of traps. In addition, nine other Tomahawk traps (model 108: 80 × 25 × 30 cm; Tomahawk Live Trap Co.) were also placed at various locations within the trapping grid to sample medium-sized mammals, primarily raccoons (*Procyon lotor*).

Traps were opened twice weekly from 1 June to 20 August, 1993. Sherman traps were baited with a small piece of peanut butter sandwich at approximately 1600 hr and checked and closed before 0900 hr the following morning to capture the nocturnal mice. Chipmunk traps were baited with a small piece of peanut butter sandwich and sunflower seeds before 0900 hr and were checked and closed before 1500 hr each day. Medium mammal traps were baited with sardines and traps were left open for the duration of each week's trapping; they were checked twice daily. All captured animals were taken to a field research laboratory for processing.

All captured animals were anesthetized with a 1:1 volume mixture of acepromazine maleate (PromAce, Fort Dodge Laboratories, Iowa, USA) and ketamine (Ketaset, Fort Dodge): 0.01 ml for mice, 0.07 ml for chipmunks and 1 to 2 ml for raccoons.

While anesthetized, each animal was checked for previous marking. The captured animal's sex and weight were recorded. An unmarked animal was given a number by either toe clipping (mice) or an ear tag (chipmunks and raccoons; National Band and Tag Co., Newport, Kentucky, USA). Chipmunks and mice were thoroughly examined for the presence of ticks, which were removed and placed into 70% ethanol for later identification.

Ear punch biopsies were taken from unmarked mice and chipmunks using the method of Sinsky and Piesman (1989). Raccoons were sampled using a Keyes biopsy punch (National Band and Tag Company, Newport, Kentucky, USA) to acquire a piece of ear tissue. Previously marked animals were biopsied again if the time interval between samples was more than 4 wk. A few chipmunks which had probably lost their ear tags during the study were retagged and biopsied at the time of recapture, which may have resulted in inadvertent repeat sampling of this species.

All captured raccoons and selected chipmunks and mice were placed into cages: 28 × 15 × 13 cm for mice, 41 × 30 ×25 cm for chipmunks, and 76 × 61 × 61 cm for raccoons. These cages were placed over pans of water for 72 hr. Animals were given water and laboratory mouse chow ad libitum (chipmunks and mice) or water and commercial dog food (raccoons) ad libitum during their confinement. After examination, any biopsy, and confinement, all animals were released at their location of capture.

All ticks which fed on these animals and fell into the water pans were collected and placed into vials which contained a moisture retentive plaster and charcoal mixture (Fish and Daniels, 1990). Vials were kept in dessicators maintained at 85% relative humidity by a solution of saturated potassium phosphate (Winston and Bates, 1960). The ticks were kept at 25 to 30 C on a 15:9 light dark cycle until examination. Ticks were removed from their vials 120 days after collection and placed into vials of 70% ethanol. Due to excessive fungal growth in some samples, approximately 5% of these ticks were lost to contamination.

All ear punch biopsies were rinsed in 70% ethanol and placed into 2 ml of BSK II medium fortified with 6% rabbit serum (Sigma Chemical Co., St. Louis, Missouri, USA). This medium is effective at detecting *Borrelia burgdorferi* in culture samples, even those with low initial spirochete numbers (Pollack et al., 1993). All samples were then placed into a 37 C air incubator. Aliquots from each sample were examined after 2 and 4 wk of incubation. A wet mount was prepared from each sample and examined by dark field microscopy at 400 × magnification. Approximately 20 microscopic fields from each wet mount were examined for thin, flexible, helical cells of 4 to 30 μm in length as described by Johnson et al. (1984). All spirochetes found by this technique were consid-

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ered *B. burgdorferi*, which has previously been serologically identified at this study site (Nelson et al., 1991). To our knowledge, no spirochetes other than *B. burgdorferi* have ever been isolated from the small mammals of Castle Rock State Park.

The number of chipmunks and mice present in the trapping grid at each trapping period was estimated using the Lincoln-Petersen Estimate recommended by Mares et al. (1981):

$$N = (M \times n)/m$$

Where N is the total number of animals in the population at a given time, M is the total number of marked animals in the population at that time, n is the total number of unmarked animals caught during the trapping session at that time and m is the number of marked animals recaptured in the same trapping session.

Mean intensities of larvae and nymphs from each trapping date were analyzed for differences between host species using the Mann Whitney *U*-test (Snedecor and Cochran, 1967). Differences in prevalence of larvae and nymphs on mice and chipmunks captured at each date were analyzed using a Fisher's exact test (Fleiss, 1981).

Overall differences in the intensity and prevalence of larvae and nymphs between chipmunks and mice were compared at each date using a Wilcoxon paired sample test (Ghent, 1974a). Correlation of intensity and prevalence between species was evaluated using Spearman's *rho*, a nonparametric correlation coefficient (Ghent, 1974b).

Differences among molting rates of larvae which fed upon the three host species were analyzed using a Chi-square test for 2 × 2 tables with Yates correction (Fleiss, 1981). Sample size was inadequate to test for differences among molting rates for nymphs.

Data from BSK culture samples were divided into subsets for June, July, and August, since animals were sampled approximately every month. Differences between *B. burgdorferi* prevalence for mice and chipmunks for each month were analyzed using a Fisher's exact test for 2 × 2 tables (Fleiss, 1981). Trends in *B. burgdorferi* prevalence for each host species over the course of the summer were analyzed using a Mantel-Haenszel Chi-square test for trend (EpiInfo, Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

RESULTS

Mouse population density in the study site was very high throughout the study period, typically almost double the numbers of chipmunks (Table 1). Estimated mouse populations rose steadily through early August while chipmunk captures rose abruptly in late June.

TABLE 1. Total captured and estimated populations of *Tamias striatus* and *Peromyscus leucopus* in a 54,000-m^2 trapping grid in Castle Rock State Park during summer, 1993.

Date	Total mice captured	Estimated mouse population	Total chipmunks captured	Estimated chipmunk population
10 June	28	112	18	51
16 June	35	115	19	61
23 June	31	115	18	78
30 June	38	110	35	126
7 July	39	135	25	90
14 July	27	165	27	83
22 July	46	194	20	96
28 July	53	207	20	108
3 August	58	232	27	97
11 August	54	225	19	121
18 August	40	239	17	133

Our trapping area was not large enough to adequately assess raccoon population density. Raccoons captured had a larval prevalence of 96% and a mean intensity of 19.3 larvae/animal. Nymphal prevalence was also 96% with a mean intensity of 1.9 nymphs/animal. However, all of these results were skewed by one juvenile raccoon which was infested with 136 larvae and six nymphs. However, raccoons did not occur in our study area in high enough numbers (n = 23 for the whole summer) to be included in the subsequent comparisons of prevalence and intensity of infestation.

Larvae were consistently more prevalent on mice than on chipmunks over the duration of the study, differing significantly ($P < 0.01$) in late July and mid-August. Intensity of larvae was consistently greater on mice than on chipmunks, often 3- to 4-fold (Fig. 1b). Over the course of the summer, the overall intensity of larvae was significantly greater for mice than chipmunks ($P < 0.01$). Prevalence and intensity of larvae on mice were both significantly temporally correlated with prevalence and intensity of larvae on chipmunks (prevalence *rho* = 0.521, $P < 0.05$; intensity *rho* = 0.854, $P < 0.01$).

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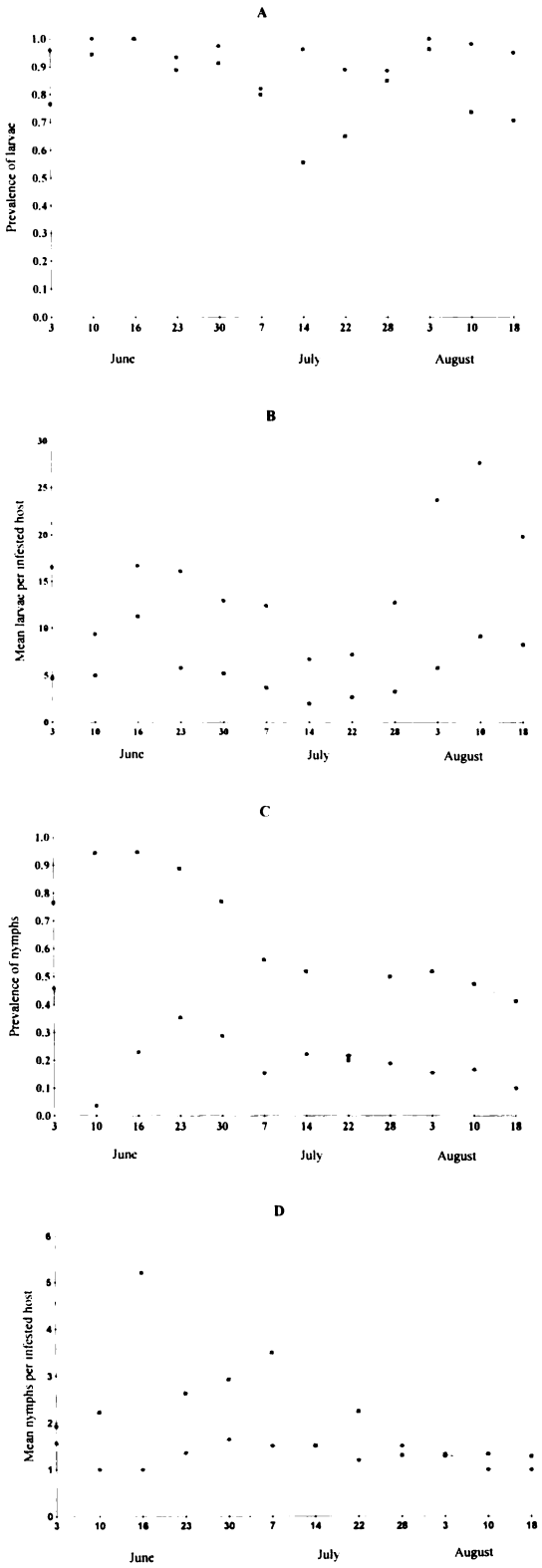

In contrast to larvae, nymphs were more prevalent ($P < 0.01$, Wilcoxon rank test) on chipmunks than on mice (Fig. 1c). Prevalence was significantly ($P < 0.05$, Fischer exact test) greater on chipmunks than on mice at each trapping date over almost the entire study period. The overall intensity of nymphs was also greater on chipmunks than on mice ($P < 0.01$, Wilcoxon rank test), and the differences were significant ($P < 0.05$, Fischer exact test) during the peak nymphal activity of June (10 to 23 June) and early July (7 July). Also, no temporal correlation was found between either prevalence or intensity of nymphs on mice and chipmunks (prevalence *rho* = 0.315, $P > 0.10$; intensity *rho* = 0.179, $P > 0.50$).

There were no differences ($P = 0.48$) in molting success of larvae which fed on chipmunks (n = 19 chipmunks sampled; 42(35%) of 120 larvae molted) and those which fed on mice (n = 25 mice sampled; 131 (39%) of 134 larvae molted). However, larvae which fed on chipmunks and those which fed on mice were both significantly ($P < 0.05$ and $P < 0.01$, respectively) more likely to molt into nymphs than larvae which fed on raccoons (n = 23 raccoons; 107 (24%) of 445 larvae molted).

We cultured and examined 130 ear punch biopsies from chipmunks, 261 biopsies from mice, and 19 biopsies from raccoons. As no ear punch samples obtained from raccoons were positive for spirochetes, raccoons do not appear capable of supporting spirochete infection and therefore were excluded from further analysis. Throughout the summer, *B. burgdorferi* infections were always more prevalent in chipmunks (34 (26%) of 130 sam-

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FIGURE 1. Prevalence and mean intensity of larvae and nymphs infesting mice (●——●) and chipmunks (■– – –■) in Castle Rock State Park, Illinois, June–August, 1993. A. Prevalence of larvae; B. Mean number of larvae per infested host; C. Prevalence of nymphs; D. Mean number of nymphs per infested host.

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ples positive for whole summer) than in mice (40 (15%) of 261 samples positive for whole summer). However, this difference was only significant ($P = 0.02$) in the month of June, when prevalence of *B. burgdorferi* in chipmunks (12 (21%) of 58 June samples positive) was more than three times that in mice (six (6.5%) of 92 June samples positive). Significant tends towards increased prevalence of infection over the course of the summer were present in both the mouse ($P < 0.01$) and chipmunk ($P < 0.05$) populations.

DISCUSSION

The results of this study provide further evidence for the key role that chipmunks play in the epizootiology of Lyme borreliosis in northwest Illinois. Chipmunks serve as important hosts for *I. scapularis* larvae and nymphs and as significant reservoirs for the spirochetes in this region. Mannelli et al. (1993) found that chipmunks played a major role as hosts for both larvae and nymphs in northwest Illinois. We found that chipmunks had a major role as hosts for nymphs, but were less important as hosts for larvae. The discrepancy in the findings of the present study and those of Mannelli et al. (1993) can be explained by examining differences in the populations of these two rodent host species between 1991 and 1993. In 1991, a year of severe drought, population density of mice was very low and the number of mice captured was approximately equal to the number of chipmunks captured (Mannelli et al., 1993). In contrast, the capture rate of mice in 1993 was almost twice that of chipmunks.

Large changes in population density are not uncommon for *P. leucopus* populations (Wolff et al., 1988). Oscillations of 7- to 15-fold over a period of several years have been reported (Grant, 1976). Population declines are usually attributed to a combination of decreased reproductive performance and high mortality and dispersal (Wolff, 1992). Chipmunks have lower dispersal and mortality rates than mice (Tryon and Snyder, 1973), so chipmunk populations are more stable over time (Grant, 1976).

It has been previously demonstrated (Mannelli et al., 1993) that larval ticks will feed as readily on chipmunks as on mice. Further, we found that there was no difference in the ability of larval ticks to molt to nymphs whether they feed on chipmunks or mice. Thus, mice and chipmunks appeared equally effective and accessible as hosts for larval ticks. Hence, in northwest Illinois, when mouse populations are low, chipmunk populations can serve as a major host for larvae. When mouse populations increase, as occurred by the time of the present study, larvae again can feed on the more prevalent host species. The finding that the intensity of larvae on mice and chipmunks were correlated is evidence that the proportion of larvae feeding on a given host species is directly related to the density of that species, when there is no difference in host preference by larval ticks. The presence of chipmunks in an ecosystem lends stability to a focus of Lyme borreliosis by providing a stable alternative host population for the vector at times when the primary host (mouse) population is in decline.

Chipmunks can also serve as a significant reservoir for *B. burgdorferi*, particularly during times when mouse populations are reduced. At such times, chipmunks become the major hosts for larvae (Mannelli et al., 1993) and thus also the primary reservoir for spirochetes, as larvae can acquire spirochetes from infected chipmunks (McLean et al., 1993). In our study area, prevalence of spirochete infection in chipmunks was similar to the prevalence in mice during August when larvae are most active. In addition, chipmunks had a significantly higher prevalence of infection in June when nymphs were active, and thus could serve as a major source of infection for adult ticks.

This high prevalence of infection for chipmunks in early summer was probably a result of two factors. First, chipmunks

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live much longer, often 3 to 4 yr (Tryon and Snyder, 1973), than mice (usually 1 yr) (Wolff et al., 1988) and can apparently support *Borrelia burgdorferi* infections indefinitely (McLean et al., 1993); therefore enzootic infections within the chipmunk population will carry over from one year to the next. Further, as the primary nymphal hosts, chipmunks are likely to encounter more infected nymphs than mice; thus, the prevalence of infection during nymphal questing should be expected to rise at a higher rate in the chipmunk population. As the major nymphal host, chipmunks are more likely to acquire nymphal infestation when nymph populations are low, either from overwintering nymphs in early spring or from recently molted nymphs in late spring; hence, they are more likely to encounter infected nymphs early in the year.

Indeed, nymphs, unlike larvae, apparently have some degree of host preference in their feeding behavior (see also Mannelli et al., 1993; Godsey et al., 1987) in the upper midwestern U.S. Also, the intensities of nymphs on chipmunks and mice were not correlated; intensity of infestation on mice was relatively constant while intensity of infestation on chipmunks varied greatly. Thus mice may be exposed to nymphs at a constant rate; nymphs resulting from detachment of engorged larvae from mice in their nests probably are the major source of infestation (Mather and Spielman, 1986). Conversely, intensity of chipmunk infestation may be dependent on numbers of nymphs questing in the environment.

If this is indeed the case, then the relatively high prevalence and intensity of nymphs on chipmunks is evidence for a reduced significance of diurnal detachment of mouse-fed larvae (Mather and Spielman, 1986) in our ecosystems. Either diurnal detachment for mice occurred at a very low rate in our study site, or mice in our study area preferentially occupied arboreal nests and fed larvae simply fell to the forest floor. Individual mice frequently change their nests from ground to arboreal sites in response to food availability (Dooley and Deuser, 1990) and this may account for the large number of nymphs on chipmunks.

Studies from the coastal northeastern United States have implicated the white footed mouse as the major host for immature *I. scapularis* and the major reservoir for *B. burgdorferi* (Mather et al., 1989). The results presented here provide additional support for the theory that in some regions of the midwestern U.S. the eastern chipmunk also plays a major role in the enzootic cycle of Lyme borreliosis.

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