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Source: Journal of Wildlife Diseases, 26(3) : 339-345

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.3.339

Journal of Wildlife Diseases, 26(3), 1990, pp. 339–345

THE ROLE OF MEDIUM-SIZED MAMMALS AS RESERVOIRS OF *BORRELIA BURGDORFERI* IN SOUTHERN NEW YORK

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ABSTRACT: The ability of raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*) and opossums (*Didelphis virginiana*) to serve as reservoirs of *Borrelia burgdorferi*, the spirochetal agent of Lyme disease, was compared with that of white-footed mice (*Peromyscus leucopus*). Twenty-eight (28) medium-sized mammals and 34 white-footed mice were captured in Westchester County, New York (USA) in summer 1986. Animals were caged over pans of water for 1 to 2 days to recover engorged tick larvae (*Ixodes dammini*) that detached from the hosts after feeding. With the exception of mice, numbers of engorged tick larvae recovered exceeded those counted during initial examinations of the hosts by 30% (opossums) to nearly 90% (raccoons). Newly-molted nymphal ticks derived from the engorged larvae were examined for the presence of spirochetes by darkfield microscopy. Percentage infection was 5% ($n = 22$) for ticks from skunks and 14% ($n = 191$) for ticks from raccoons. None of 24 nymphs from larvae that fed on opossums survived long enough for spirochete examination. By comparison, 40% ($n = 72$) of nymphs from larvae which fed on white-footed mice were infected. Of the individual hosts from which molted nymphs had fed as larvae, 67% of mice, 33% of skunks, and 55% of raccoons produced spirochete-positive ticks.

Key words: *Borrelia burgdorferi*, *Ixodes dammini*, Lyme disease, reservoirs, xenodiagnosis, arachnida, field study, ticks.

INTRODUCTION

The maintenance of *Borrelia burgdorferi* in nature has been a subject of investigation since the discovery of the spirochetal etiology of Lyme disease (Burgdorfer et al., 1982; Anderson et al., 1983; Bosler et al., 1983; Steere et al., 1983). A variety of mammal and bird species has been implicated as reservoirs of infection for the tick vector *Ixodes dammini*. For most, the evidence is based upon the recovery of *B. burgdorferi* from host blood and other tissues, from feeding ticks, or the presence of *B. burgdorferi*-specific antibodies in host blood (Anderson et al., 1983; Bosler et al., 1983; Anderson and Magnarelli, 1984; Bosler et al., 1984; Anderson et al., 1985, 1986a; Magnarelli et al., 1984, 1988; Weisbrod and Johnson, 1989). Thus far, only a few small mammal species (Mather et al., 1989), including the white-footed mouse, *Peromyscus leucopus* (Levine et al., 1985; Donahue et al., 1987), have been shown empirically to be reservoir competent in nature. *Peromyscus leucopus* also has been shown to be a principal host for immature *I. dammini* (Piesman and Spielman, 1979; Anderson and Magnarelli, 1980; Main et al., 1982), suggesting that infectious nymphs, which constitute the greatest risk to humans (Steere et al., 1978; Steere and Malawista, 1979), are derived mainly from a single host species (Mather et al., 1987).

Medium-sized mammals also have been shown to be significant hosts of immature *I. dammini* (Carey et al., 1980; Main et al., 1982; Magnarelli et al., 1984; Fish and Dowler, 1989). Raccoons (*Procyon lotor*), skunks (*Mephitis mephitis*), and opossums (*Didelphis virginiana*) have been implicated as reservoirs of the agent for Lyme disease by virtue of either positive blood cultures or positive serology (Anderson et al., 1983, 1986b). However, a direct measure of reservoir status of hosts is preferable to indirect evidence from serology or culture. A xenodiagnostic technique has been described which demonstrates vector infectivity by feeding uninfected larval ticks on candidate hosts and then examining the ticks' body contents for spiro-

chetes after they have molted into nymphs (Levine et al., 1985; Donahue et al., 1987). This technique is not only inexpensive, but is also a more realistic assessment of reservoir competence because it emulates all of the natural events that would lead to the production of a host-seeking infectious nymph in nature.

The purpose of this study was to investigate the ability of these medium-sized mammalian species to infect *I. dammini* larvae with spirochetes. Thus, their roles in producing infectious nymphs and, therefore, their contribution to the maintenance of *B. burgdorferi* in nature could be determined.

MATERIALS AND METHODS

Study site

Field work was conducted at the Louis Calder Conservation and Ecology Study Center of Fordham University, located 28 km north of New York City (41°8′N, 73°48′W in the village of Armonk, Westchester County, New York 10504 USA), where Lyme disease is endemic (Williams et al., 1986). The habitat is characteristic of mature and late seral eastern deciduous woodland with a mix of oaks (*Quercus* spp.), sugar maple (*Acer saccharum*), gray birch (*Betula populifolia*) and tulip trees (*Liriodendron tulipifera*), as well as a sparse understory of shrubs.

Animal trapping and handling

All mammals were live-trapped between 6 August and 11 September 1986, during the summer peak of larval tick abundance (Piesman and Spielman, 1979; Daniels and Fish, 1990). Because of their demonstrated ability to serve as reservoirs for *B. burgdorferi*, white-footed mice served as positive controls with which to compare data from medium-sized mammals. Mice were trapped for 12 nights (471 trap nights) using small (5.1 × 6.4 × 16.5 cm) Sherman traps (H.B. Sherman Traps, Inc., Tallahassee, Florida 32301, USA) which were baited with peanut butter and oats. Medium-sized mammals were trapped for 13 nights (170 trap nights) using #205 (66.0 × 22.9 × 22.9 cm) and #207 (81.3 × 25.4 × 30.5 cm) tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin 54487, USA) baited with dry dog food.

All captured mammals were brought to the Medical Entomology Laboratory (New York Medical College, Armonk, New York 10504, USA), anesthetized either by inhalation of ether (mice) or intramuscular injection of ketamine hydrochloride (1 mg/kg body wt.; Ketaset, Bristol Laboratories, Syracuse, New York 13201, USA), and examined for the presence of ticks. Mice were examined for approximately 3 to 4 min and larger mammals were examined for approximately 15 min. Mammals were then sexed, toe-clipped (mice) or ear-tagged (National Band and Tag Co., Newport, Kentucky 41071, USA), and held in wire cages suspended over pans of water for 24 to 48 hr. Food and water were supplied ad libitum.

Tick collections

Engorged tick larvae and nymphs which dropped from the hosts and into the water pans were recovered daily, counted and identified to species. All engorged *I. dammini* larvae were rinsed thoroughly with distilled water, blotted dry on absorbent paper toweling, and placed in vials containing a mixture of hardened plaster of Paris and charcoal, which retains moisture when dampened. Vials were capped with 4 × 4 cm swatches of organdy material and placed in glass chambers in which relative humidity was maintained at approximately 95% with a solution of saturated KH_2PO_4 (Winston and Bates, 1960). Ticks were maintained at approximately 20 C until molting was completed.

Tick dissections

Newly-molted tick nymphs were removed from the vials and dissected. Body contents were extruded onto a microscope slide containing 1 drop of phosphate-buffered saline (FTA-ABS PBS, Zeus Scientific, Inc., Raritan, New Jersey 08869, USA), covered with a cover slip, and examined for the presence of spirochetes by darkfield microscopy (Anderson et al., 1983). We used darkfield microscopy for budgetary reasons, noting that the fluorescent antibody technique does not improve sensitivity (Piesman et al., 1986a) and the frequently used monoclonal antibody H5332 (Barbour et al., 1983) is not specific for *B. burgdorferi*. Darkfield microscopic examinations were initiated on 7 October and conducted twice weekly, on average, through mid-December. The number of ticks which was positive for the presence of spirochetes was noted; the proportion of ticks ([number positive/total number examined] × 100) infected as a result of feeding on a particular mammal species was calculated to serve as an index of that species' ability to act as a reservoir.

Unfed tick larvae were obtained from the study area to serve as negative controls. Questing larvae were collected on 12 September 1986 by pulling a 1 m² piece of white corduroy cloth along the ground and over low vegetation (Milne,

TABLE 1. *Ixodes dammini* larvae collected from different mammal species in southern New York.

Host species	Number examined	Number (%) with larvae	Number engorged larvae	Mean ticks (SD) per host	Mean % molted
Peromyscus leucopus	34	32 (94)	256	8 (3)	24
Procyon lotor	16	15 (94)	2,168	136 (12)	33
Mephitis mephitis	7	6 (86)	298	43 (8)	19
Didelphis virginiana	6	6 (100)	323	54 (6)	4

1943). These larvae were maintained at approximately 95% relative humidity until dissected and examined for the presence of spirochetes by darkfield microscopy.

Statistical analysis

Analyses of proportional data were performed by analysis of variance (ANOVA) after testing for normality. Because data (proportion of larvae molting to nymphs and proportion of ticks infected with spirochetes) were normally distributed as indicated by the Kolmogorov-Smirnov Test for Goodness of Fit at a significance level of $P < 0.05$ (Sokal and Rohlf, 1981), they were not transformed. Chi-square analysis of the numbers of larvae molting to nymphs was performed to determine if molting success was independent of host species.

RESULTS

Sixty-three mammal captures, including recaptures, were examined for ticks (Table 1). *Ixodes dammini* was the predominant tick species found on mammals, comprising 99% of the ticks collected. *Ixodes cookei* and *Dermacentor variabilis* comprised the remaining 1%, but because of their infrequent occurrence, they were deemed unimportant for this study and are not discussed further.

Prevalence of *I. dammini* larvae, expressed as the percentage of captured host individuals that were parasitized (Margolis et al., 1982), is presented in Table 1. Engorged larvae were recovered from all of the medium-sized mammals that had ticks and from all but four mice.

Except for mice, initial counts of larvae infesting hosts indicated consistent undercounting of ticks. For mice, 267 larvae were counted for 34 captures, while only 256 larvae were collected from the water pans. But only 11% (n = 2,168) of the larvae on raccoons, 13% (n = 298) of the larvae on skunks, and 70% (n = 323) of the larvae of opossums were counted prior to suspending animals over pans of water.

The number of engorged larvae collected from individual animals ranged from two to 43 per mouse, 27 to 503 per raccoon, four to 161 per skunk, and 16 to 117 per opossum. Mean intensity, expressed as the mean number of larvae per host examined (Margolis et al., 1982), ranged from eight (mouse) to 136 (raccoon) (Table 1).

Average molting success, expressed as the percentage of engorged larvae molting to nymphs per host, was 24% (SD = 5.3) for mice (n = 28), 33% (SD = 4.4) for raccoons (n = 15), 19% (SD = 4.3) for skunks (n = 6), and 4% (SD = 2.5) for opossums (n = 6). There was no significant effect of host species on the proportion of larvae molting to the nymphal stage (one-way ANOVA; $F = 2.07$, $P = 0.12$). However, a comparison of the mean number of larvae that fed on white-footed mice and molted to nymphs to that for opossums indicated a significant difference ($\chi^2 = 50.3$, $P < 0.05$, 1 df). Thus, while the overall effect of host species on molting success was insignificant due to the large variance within a species, there is evidence that larvae which fed on opossums were less likely to molt than were larvae which fed on white-footed mice. Molted nymphs were derived from 15 (54%) mice, 15 (100%) raccoons, four (67%) skunks, and three (50%) opossums.

The number of newly-molted nymphs per host that were examined for the presence of spirochetes varied from one to 23,

TABLE 2. Prevalence of spirochetes in nymphal *Ixodes dammini* collected as larvae from mammals in southern New York.

Host species	Number of ticks examined	Number of hosts	Number of ticks positive	% positive	Range
Peromyscus leucopus	72	12	29	40	0–100
Mephitis mephitis	22	3	1	5	0–5
Procyon lotor	191	11	26	14	0–50
Flat larvae	48	—	0	0	0

depending upon the number of engorged larvae recovered and molting success. The total number of nymphs examined from medium-sized mammals was 213:191 from 11 different raccoons and 22 from three skunks (Table 2). Although 24 of the engorged larvae recovered from opossums successfully molted to nymphs, all died within a few days of molting and before they could be examined for spirochetes. Of the 191 total nymphs examined from raccoons, 26 (14%) were positive for the presence of spirochetes while only one (5%) of 22 nymphs from skunks was positive (Table 2). By comparison, 40% ($n = 72$) of the newly-molted nymphs from 12 mice were positive. None of the 48 larvae collected by drag sampling to serve as negative controls was positive for the presence of spirochetes. One-way ANOVA indicated a significant effect of host species on the percentage of ticks infected with spirochetes ($F = 4.06$, $P = 0.029$).

Within a species, the percentage of infected ticks from a single host ranged from 0 to 100% for white-footed mice, 0 to 50% for raccoons, and 0 to 5% for striped skunks (Table 2). Among individual hosts from which molted nymphs were examined, 67% ($n = 12$) of mice, 55% ($n = 11$) of raccoons, and 33% ($n = 3$) of skunks produced spirochete-positive ticks.

DISCUSSION

Ixodes dammini larvae were present on 59 of 63 mammals captured during this study, suggesting that all mammals at the study site probably were exposed to larval ticks. Immature *I. dammini* exhibit a broad host range (Anderson and Magnarelli, 1980; Main et al., 1982; Fish and Dowler, 1989). Consequently, the potential for larval infestation is high across species, and comparisons of reservoir competence are important.

Discrepancies between the number of larvae initially counted on hosts and the higher number subsequently recovered in water pans suggest that conclusions about host importance, as determined by the observed numerical abundance of attached ticks, may be subject to error. This is less of a concern with white-footed mice, for which nearly all larvae were counted, than with larger mammal species whose relative importance as hosts for larval deer ticks is likely to be seriously underestimated. Undercounting resulted largely from difficulties in seeing the larvae during whole-body examinations of hosts, particularly skunks and raccoons, which have a relatively large body surface area and dark, dense fur. Efforts to assess the importance of different mammal species as hosts of immature deer ticks should include holding mammals for several days and collecting replete ticks rather than relying upon examinations for attached ticks at the time of capture.

Average molting success was statistically the same for larvae recovered from all four mammal species. However, the relative contribution each species makes to the overall tick population will be a function of host species' abundance, as well as the number of ticks per host.

Although all of the medium-sized mammal species we examined were parasitized

by larval *I. dammini*, their capacity to serve as sources of spirochete infection varied significantly. Donahue et al. (1987) noted that golden Syrian hamsters were less able to transmit spirochetes to *I. dammini* larvae than were white-footed mice because of host immunity acquired against the vector. Host immune responses to ixodid tick feeding are well-known and may result in tick rejection, lower engorged weight of feeding ticks, decreased fecundity of female ticks, and prolonged feeding duration (Randolph, 1979; Willadsen, 1980; Brown et al., 1984; Shapiro et al., 1987). The high mortality of nymphs that fed as larvae on opossums may indicate that these mammals exhibit such an immune response and, therefore, would be a poor source of infected ticks.

Our evaluation of reservoir competence among the mammal species in this study depends upon the assumptions that all animals were infected with *B. burgdorferi* and that all recovered larvae were uninfected prior to feeding. Nymphal *I. dammini* are abundant on all medium-sized mammals at this site (Fish and Dowler, 1989) and the spirochete infection rate in questing nymphs is 30% ($n = 104$; D. Fish, unpublished data). Although we did not attempt to demonstrate host infection, it is not likely that any of these mammals could have avoided an infectious tick bite. Transovarial transmission of *B. burgdorferi* to larval *I. dammini* is rare in nature (<2%) (Steere et al., 1983; Piesman et al., 1986b; Magnarelli et al., 1987) and the absence of spirochetes in the 48 larvae we examined confirms our assumption. Thus, spirochetes observed in ticks removed from mammals were acquired during larval feeding.

The only mammals previously shown to be reservoir competent for Lyme disease are white-footed mice, *P. leucopus* (Levine et al., 1985; Donahue et al., 1987), chipmunks, *Tamias striatus*, and meadow voles, *Microtus pennsylvanicus* (Mather et al., 1989). Despite our finding that raccoons and skunks are competent reservoirs, the difference in host species' ability to infect larvae with spirochetes indicates that white-footed mice are relatively more efficient in transmitting infection than are medium-sized mammals. However, when the mean number of ticks per host also is considered, findings indicate that a single raccoon produces as many infected nymphal *I. dammini* as six mice, while one skunk is approximately equivalent to one mouse in its ability to produce infected nymphs. The overall significance of these host species' differences with respect to the ecology of Lyme disease is not known. The role each species plays in maintaining *B. burgdorferi* in nature will depend on the population density of the host species, how host density varies temporally when different tick stages are active, prevalence and intensity (Margolis et al., 1982) of *I. dammini* for each species, and even habitat type. For example, Fish and Dowler (1989) noted that medium-sized mammals may be less important hosts of immature *I. dammini* in forested areas where *P. leucopus* are abundant, but more important in suburban residential areas where the density of medium-sized mammals is higher but that of *P. leucopus* may be lower.

Donahue et al. (1987) reported that for their sites in Massachusetts, no larval cohort that fed on *P. leucopus* 2 to 3 wk after nymphal attachment was <70% infected. By contrast, our data indicate that mice infected only about 40% of the larvae that successfully molted to nymphs. But, Donahue et al. (1987) observed that transmission of spirochetes declined to about 50% of the larvae that fed 9 wk after infection of the host by nymphs. The present study was conducted during August and September, approximately 8 to 12 wk after *I. dammini* nymphal populations peak in Westchester County (Fish and Dowler, 1989). Thus, differences in infectivity of mice reported in this study and that reported by Donahue et al. (1987) may reflect a natural decline in host ability to transmit spirochetes. However, our data more closely

reflect events in the field because they were obtained during the period of peak larval abundance in nature.

Although previous studies have emphasized the role of *P. leucopus* as the primary reservoir of *B. burgdorferi* (Levine et al., 1985; Donahue et al., 1987; Mather et al., 1989), this study demonstrates that larger mammals also serve as reservoirs of infection. In fact, the spectrum of potential reservoirs may include additional mammalian, as well as avian, species (Anderson et al., 1986b; Battaly et al., 1987). We suggest that the maintenance of *B. burgdorferi* in nature, and consequently the epizootiology of Lyme disease, is more complex than previously thought, and control strategies directed toward a single reservoir species (Mather et al., 1987) may be unsuccessful in southern New York.

ACKNOWLEDGMENTS

We wish to thank J. Zultowsky, R. Klaiman, M. Merrifield, and J. Gutmann for their technical assistance. We also thank Richard C. Falco for helpful comments on a previous draft of the paper. Research was supported by the Harold G. and Leila Y. Mathers Charitable Foundation, Colin and Barbara Offenhartz, and an anonymous donor.

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Received for publication 19 June 1989.