BioOne COMPLETE

DURATION OF BORRELIA BURGDORFERI INFECTIVITY IN WHITE-FOOTED MICE FOR THE TICK VECTOR IXODES SCAPULARIS UNDER LABORATORY AND FIELD CONDITIONS IN ONTARIO

Authors: Lindsay, L. Robbin, Barker, Ian K., Surgeoner, Gordon A., McEwen, Scott A., and Campbell, G. Douglas

Source: Journal of Wildlife Diseases, 33(4) : 766-775

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.4.766

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Journal of Wildlife Diseases, 33(4), 1997, pp. 766–775
© Wildlife Disease Association 1997

DURATION OF *BORRELIA BURGDORFERI* INFECTIVITY IN WHITE-FOOTED MICE FOR THE TICK VECTOR *IXODES SCAPULARIS* UNDER LABORATORY AND FIELD CONDITIONS IN ONTARIO

L. Robbin Lindsay,[1] Ian K. Barker,[2] Gordon A. Surgeoner,[1] Scott A. McEwen,[3] and G. Douglas Campbell[4]

[1] Department of Environmental Biology, Ontario Agricultural College, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[2] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[3] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[4] Canadian Cooperative Wildlife Health Centre, Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT: The duration of *Borrelia burgdorferi* infectivity in white-footed mice (*Peromyscus leucopus*) experimentally inoculated or infested with infected *Ixodes scapularis* nymphs was evaluated. Infectivity was assessed by infesting these mice with unfed *I. scapularis* larvae at 7, 21, 35 and 49 days post-inoculation (DPI) or post-infestation (PI). At 7 DPI, *B. burgdorferi* was transmitted from 18 of 24 syringe-inoculated mice and all three tick-infected mice to *I. scapularis* larvae which fed upon them. However, at 21, 35 and 49 DPI, significantly fewer mice were infective. *Borrelia burgdorferi* was isolated from tissues of 14 of 22 syringe-inoculated mice about 56 DPI, and from all three tick-infected mice. However, the level of agreement between xenodiagnosis and bacterial culture was no greater than would be expected by chance alone. We also determined if *B. burgdorferi* infectivity of mice varied in relation to periods of tick feeding in the field. White-footed mice were trapped during April, July and August 1993 from two habitats on Long Point peninsula (Ontario, Canada), where *B. burgdorferi* is endemic. Mice from each habitat were infested with laboratory-reared *I. scapularis* larvae. Ticks from each mouse were subsequently examined by immunofluorescent assay for *B. burgdorferi* infection and mice were cultured for *B. burgdorferi*. None of 3577 *I. scapularis* larvae fed on 62 mice captured within the cottonwood dune habitat were infected with *B. burgdorferi*, although it was isolated from six of these mice. Within the maple forest habitat, 0/24, 8/21 (38%) and 1/21 (5%) mice transmitted *B. burgdorferi* to *I. scapularis* larvae during April, July and August, respectively. Most mice from the maple forest with *B. burgdorferi*-positive tissues (14/21) were collected during July, although the level of agreement between xenodiagnosis and tissue culture was poor. Because *B. burgdorferi* infectivity in mice appears to be of short duration, overwintered *I. scapularis* larvae and nymphs may have to feed upon infected hosts at the same time of year in order for a cycle of *B. burgdorferi* infection to be maintained on Long Point. Infected *I. scapularis* nymphs, rather than persistently infected vertebrate hosts, likely serve as the overwintering "reservoir" for *B. burgdorferi* on Long Point.

Key words: *Borrelia burgdorferi*, epidemiology, infectivity, *Ixodes scapularis*, *Peromyscus leucopus*.

INTRODUCTION

Lyme borreliosis is an infectious disease transmitted mainly by ticks of the *Ixodes ricinus* group from various reservoir hosts, including numerous species of birds (Battaly and Fish, 1993) and mammals (Anderson, 1988). White-footed mice (*Peromyscus leucopus*, herein designated "mice") are considered as primary reservoir hosts for *Borrelia burgdorferi* in the northeastern and mid-western USA (Levine et al., 1985; Godsey et al., 1987) and the principal tick vector is the black-legged tick, *Ixodes scapularis*, formerly *I. dammini* (Oliver et al., 1993). *Borrelia burgdorferi* is maintained in endemic areas when infected *I. scapularis* nymphs feed on uninfected reservoir-competent hosts (Spielman et al., 1985). These animals become infected, and when uninfected *I. scapularis* larvae feed on them, some of the ticks acquire *B. burgdorferi*. *Borrelia burgdorferi* is maintained within the wild-

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

life reservoir population through transtadial transmission in the ticks, which subsequently infect other hosts with *B. burgdorferi* when they feed as nymphs (Spielman et al., 1985). Recently, the concept has emerged that non-systemic infection with *B. burgdorferi* in the vertebrate host is significant in transmission of the agent between co-feeding *Ixodes ricinus* larvae and nymphs (Gern and Rais, 1996; Randolph et al., 1996).

Borrelia burgdorferi infectivity in naturally-infested white-footed mice has been assessed by xenodiagnosis (Mather et al., 1989a, 1989b); by culturing various tissues from mice (Anderson et al., 1985); or by using both of these techniques simultaneously (Anderson et al., 1987). Mice initially inoculated either by exposure to infected *I. scapularis* nymphs (Mather and Mather, 1990) or syringe-inoculated with cultured strains of *B. burgdorferi* (Barthold et al., 1993) are infective. Xenodiagnostic or bacterial culture techniques were then used to determine the infective status of mice. Anderson et al. (1987) proposed that after acquiring *B. burgdorferi* from infected *I. scapularis* nymphs, mice remain infected for life, because the organism can be cultured from various tissues of mice collected during the winter months (4 to 6 mo after nymphal activity has ceased).

The tick vector, *I. scapularis,* and the spirochete, *B. burgdorferi,* are endemic to the Long Point peninsula (Lake Erie, Ontario, Canada; 42°36′N, 80°5′W). Although the seasonal abundance of *I. scapularis* on various mammal species, and the seroconversion rates of these hosts have been monitored (Barker et al., 1992), the seasonal prevalence of infection with *B. burgdorferi* in populations of reservoir hosts on Long Point has not been determined. We investigated the duration of *B. burgdorferi* infectivity in mice infected in the laboratory. These observations led to a new interpretation of the pattern of vector-host-vector transmission in the epizootiology of *B. burgdorferi* infection on Long Point, compatible with the concept that co-feeding may be significant in the transmission of *B. burgdorferi* between cohorts of ticks, and maintenance of the infection in a geographic locality.

MATERIALS AND METHODS

Mice were obtained from a laboratory colony negative for antibody to *B. burgdorferi*, maintained at the University of Guelph (Guelph, Ontario, Canada) for >25 generations. Twenty-four adult mice (14 male, 10 female) each were inoculated subcutaneously between the scapulae with 0.7 to 1.6 × 10^7 *B. burgdorferi* strain LI-231. This strain originally was isolated from an engorged *I. scapularis* larva from Long Point and was confirmed as *B. burgdorferi* by indirect immunofluorescent (IFA) staining with monoclonal antibody H5332 (Barbour, 1984). Each mouse then was infested with approximately 200 to 300 unfed *I. scapularis* larvae at 7, 21, 35 and 49 days post-inoculation (DPI). These unfed larvae were hatched from eggs deposited by females that fed on a dog (*Canis familiaris*) on Long Point during April and October 1992. Three additional adult female mice each were infested with 30 *I. scapularis* nymphs, which had fed as larvae on mice that were previously syringe-inoculated with *B. burgdorferi*. These three mice then were infested with unfed *I. scapularis* larvae on the same schedule as the syringe-inoculated mice. During each infestation, mice were maintained individually in wire cages over pans of water for up to five days, and all fed larvae which dropped off were collected. Fed larvae from each mouse were stored over damp vermiculite in plastic containers (relative humidity >95%) within environment chambers at a constant 24 C and with a 12 hr light and 12 hr dark cycle.

Twelve to 14 days later smears of the midgut of fed larvae were examined for *B. burgdorferi* by IFA staining using monoclonal antibody H5332 directed against *B. burgdorferi* OspA (Lindsay et al., 1991). The intensity of *B. burgdorferi* infection in mid-gut smears was categorized into four classes: none; few (<10 organisms per entire slide well); moderate (>10 per well, but <50 organisms/field at 200×); and many (at least one field containing >50 organisms/field at 200×). To ascertain that larvae were uninfected before feeding on experimental animals, 30 unfed larvae from each *I. scapularis* egg batch used as a source of experimental larvae were examined for *B. burgdorferi* by IFA staining; no spirochetes were found. Aliquots (50 µl) of *B. burgdorferi* strain LI-231 air-dried on glass slides and fixed with

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

acetone served as positive controls. Up to 30 fed larvae from each of the four infestations on each mouse were examined for *B. burgdorferi*. Mice were considered infective if at least one *I. scapularis* larva was infected with *B. burgdorferi* after feeding. Prevalence and intensity of *B. burgdorferi* infection in *I. scapularis* larvae fed at various times following inoculation were compared statistically using the generalized linear model (Statistical Analysis System Version 6.04; SAS Institute, 1988). The general linear model also was used to compare the effect of duration of *B. burgdorferi* infection on infectivity of mice for larval ticks. Because of over dispersion of the data, square root transformations were performed prior to analysis.

After all fed larvae had dropped from mice infested at 49 DPI, mice were euthanized by inhalation of isoflurane (Aerrane®, Anaquest Division, Canada Oxygen Ltd., Mississauga, Ontario, Canada), and the urinary bladder, spleen, and left kidney were removed aseptically. The left aural pinna from each mouse was removed and immersed in 100% ethanol for 5 to 7 min. Each tissue was triturated in 1 ml of BSK II K-5 medium, modified from Barbour's (1984) medium by the addition of kanamycin and 5-fluorouracil (Johnson et al., 1984). One hundred and fifty µl of the triturate of each tissue was inoculated into a 7 ml tube of BSK II K-5 medium, incubated at 33 C and examined by dark field microscopy for spirochetes at 1, 2 and 6 wk post-inoculation (pi.). Aliquots (50 µl) from each positive culture were air-dried on glass slides, fixed in acetone for 10 min at room temperature and subjected to IFA staining to confirm that the organism isolated from mice was *B. burgdorferi*. Mice were considered infected if *B. burgdorferi* was isolated from at least one tissue. The level of agreement between the xenodiagnostic and bacterial culture techniques for detecting *B. burgdorferi* infection in mice was determined using the kappa (κ) statistic (Martin et al., 1987).

In order to examine *B. burgdorferi* infectivity of mice at various periods during the tick's life cycle, mice were captured on the Long Point peninsula during 20 and 21 April, 4 to 6 July and 18 and 19 August, 1993. These dates were selected because they are periods of the year when few nymphs are active, 3 wk after peak nymphal activity, and when newly-emerged larvae begin seeking hosts on Long Point, respectively (L. R. Lindsay, unpubl. data). Mice were collected using folding aluminum live-traps (76 × 89 × 229 mm, H.B. Sherman Traps Inc., Tallahassee, Florida, USA) placed within cottonwood (*Populus deltoides*) dune and maple (*Acer saccharum*) forest habitats on Long Point, described by Reznicek and Catling (1989). The abundance of immature *I. scapularis* on mice was about 40-fold greater in the maple forest than in the cottonwood dune (L. R. Lindsay, unpubl. data) and thus likely would influence the proportion of *B. burgdorferi*-infected mice collected.

Mice and ticks were handled in a manner similar to the laboratory studies, except that mice trapped from Long Point were infested only once with laboratory-reared larvae before they were euthanized and their organs were cultured. Since unfed *I. scapularis* larvae on Long Point rarely were infected with *B. burgdorferi* (Lindsay et al., 1991), larvae that were present on mice when they were captured also were used as xenodiagnostic ticks. Thirty fed larvae collected from each mouse were dissected and subjected to IFA staining following incubation at 24 C for 12 to 14 days; the remaining fed larvae were allowed to molt. Approximately 2 wk after the molt, up to 30 unfed nymphs from each mouse were dissected and subjected to IFA staining.

Prevalence and intensity of *B. burgdorferi* infection in ticks were determined as in the laboratory study; however, because few of the field-collected mice produced infections in feeding ticks, statistical comparisons of the effect of habitat type on these parameters were precluded. Comparison of the intensity of *B. burgdorferi* infection in fed larvae and unfed nymphs collected from the same hosts was performed using a paired *t*-test ($P \leq 0.05$) (Snedecor and Cochran, 1989). A κ value also was calculated to determine the level of agreement between xenodiagnosis and bacterial culture techniques for detection of infected mice (Martin et al., 1987).

RESULTS

The prevalence of syringe-inoculated mice that transmitted *B. burgdorferi* to feeding *I. scapularis* larvae at 7 DPI was 75% (18/24 mice) and decreased to 14% (3/22 mice) by 49 DPI (Table 1). At 7 DPI, 22% of fed larvae examined for *B. burgdorferi* infection were positive; however, by 49 DPI only about 1% of fed larvae were infected with *B. burgdorferi* (Table 1). *Borrelia burgdorferi* was observed in 29% of the fed larvae collected from mice that infected ≥one *I. scapularis* larvae at 7 DPI, whereas less than 10% of the fed larvae collected from infective mice during later infestations were positive for *B. burgdorferi* (Table 1). The proportion of larvae

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 1. Efficacy of transmission of *Borrelia burgdorferi* to *Ixodes scapularis* larvae at various times following infection of mice in the laboratory.

	Days post-inoculation			
	7	21	35	49
	Syringe-inoculated mice			
Proportion of mice infective to larvae	18/24 (75)[a]	6/23 (26)[b]	4/22 (18)[b]	3/22 (14)
Proportion of larvae infected				
All mice	158/720 (22) [114,44,0][c]	9/690 (1) [8,1,0]	11/660 (2) [11,0,0]	7/660 (1) [7,0,0]
Infective mice only[d]	158/540 (29)	9/180 (5)	11/120 (9)	7/90 (8)
	Tick-inoculated mice			
Proportion of mice infective to larvae	3/3 (100)	0/3	0/3	0/3
Proportion of larvae infected	56/90 (62) [7,25,24]	0/90	0/90	0/90

[a] Number positive for *B. burgdorferi*/number tested (percent positive).
[b] One mouse died prior to completion of the infestation.
[c] Number of infected ticks in each *B. burgdorferi* infection class [few, moderate, many].
[d] Infective mice infected at least one *I. scapularis* larva with *B. burgdorferi*.

infected at higher intensities with *B. burgdorferi* was significantly ($P = 0.001$) greater at 7 DPI compared to the other infestation dates, which were not significantly ($P = 0.2$) different from each other.

All three mice inoculated by infestation with infected *I. scapularis* nymphs transmitted *B. burgdorferi* to larvae fed at 7 DPI (Table 1). The intensity of *B. burgdorferi* infections within fed larvae was significantly ($P = 0.001$) greater when ticks were fed at 7 DPI on the tick-infected mice, compared to syringe-inoculated mice. However, mice inoculated by nymphal feeding were not infective to any *I. scapularis* larvae during the three subsequent infestation periods (Table 1).

At the termination of the experiment, *B. burgdorferi* was isolated from the tissues of 14 of 22 syringe-inoculated mice and from all three mice inoculated by *I. scapularis* nymphs (Table 2). The organism

TABLE 2. *Borrelia burgdorferi* infectivity of mice for *I. scapularis* larvae at various times after inoculation, in relation to *B. burgdorferi* culture status of mice at 56 DPI.

Mouse category	Days post-inoculation				Culture status of mice (56 DPI)
	7	21	35	49	
	Syringe-inoculated mice				
Never infective to ticks[a]	0/2[b]	0/2	0/2	0/2	(−)[c]
	0/4	0/4	0/4	0/4	(+)[d]
Infective to ticks[e]	10/10	2/10	1/10	1/10	(+)
	6/6	4/6	3/6	2/6	(−)
	2/2	0/1[f]	0/1[f]	NA[g]	NA
	Tick-inoculated mice				
Infective to ticks	3/3	0/3	0/3	0/3	(+)

[a] Mice from which infected *I. scapularis* larvae were never recovered.
[b] Number of mice infecting at least one *I. scapularis* larva/number of mice tested.
[c] *Borrelia burgdorferi* not isolated from any tissues cultured from each mouse.
[d] *Borrelia burgdorferi* isolated from at least one tissue cultured from each mouse.
[e] Mice from which at least one infected *I. scapularis* larva was recovered at some stage of the experiment.
[f] One mouse died prior to completion of the infestation at each time.
[g] Not available, due to premature death of mice; mice were not cultured.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 3. Proportion of mice collected within the maple forest habitat on Long Point (Ontario, Canada) that were infective to feeding *I. scapularis* larvae; the proportion of fed larvae or resulting nymphs that became infected with *B. burgdorferi*; and the proportion of mice from which *B. burgdorferi* was subsequently cultured, during April, July and August, 1993.

	Month of infestation			
	April	July	August	Total
Proportion of mice infective to ticks	0/24 (0)[a]	8/21 (38)	1/21 (5)	9/66 (14)
Proportion of ticks infected				
Fed larvae	0/720	76/630 (12)[b] [16,39,21][c]	5/630 (1) [3,1,1]	81/1,859 (4) [19,40,22]
Unfed nymphs	0/526	49/532 (9) [8,32,9]	10/528 (2) [4,5,1]	59/1,718 (3) [12,37,10]
Total	0/1,246	125/1,162 (11) [24,71,30]	15/1,158 (1) [7,6,2]	140/3,577 (4) [31,77,32]
Culture status of mice	6/24[d]	14/21	3/21	23/66

[a] Number of mice that infected larvae with *B. burgdorferi*/number tested (percent infective).
[b] Number of ticks infected with *B. burgdorferi*/number examined (percent infected).
[c] Number of infected ticks in each *B. burgdorferi* infection class [few, moderate, many].
[d] Number of mice positive for *B. burgdorferi*/number tested.

was recovered from 11 of 25 spleens, five of 25 bladders, two of 25 ears and one of 25 kidneys. Two syringe-inoculated mice had *B. burgdorferi* in more than one tissue. Four of the six mice which had never infected *I. scapularis* larvae had *B. burgdorferi* when cultured (Table 2). The spirochete was isolated from the tissues of 10 of 16 mice which transmitted *B. burgdorferi* to ticks during one or more of the infestations (Table 2). Only three of these 16 mice were infective for ticks at 49 DPI, and the level of agreement ($\kappa = 0.04$) between xenodiagnosis at 49 DPI and culture of tissues was no greater than that which would be expected by chance alone.

The proportion of *B. burgdorferi*-infective wild mice differed between the two habitats on Long Point. Although *B. burgdorferi* was isolated from two of 20 to 22 mice collected in each of April, July, and August in the cottonwood dune habitat, none of the fed larvae (600 to 660 per sampling interval) or resulting unfed nymphs (527 to 611 per sampling interval) exposed to these mice during each sampling interval acquired *B. burgdorferi* infections.

None of the 24 adult mice collected in the maple forest habitat during April was infective for *I. scapularis* larvae. However, during July and August, respectively, eight (38%) of 21 and one (5%) of 21 mice captured within this habitat transmitted *B. burgdorferi* to feeding *I. scapularis* larvae (Table 3). The prevalence of *B. burgdorferi* infection within fed larvae and unfed nymphs which were allowed to molt from the same cohort of fed larvae did not differ significantly ($P = 0.60$). Overall prevalence of *B. burgdorferi* in fed immature *I. scapularis* ranged from 125 (11%) of 1162 in July, to 15 (1%) of 1158 in August. However, considering only ticks from mice which were infective to at least one tick, the spirochete was observed within 29 and 31% of immature *I. scapularis* during July and August, respectively.

Borrelia burgdorferi was isolated from mice collected within the maple forest during each sampling period (Table 3). Tissues from six mice were *B. burgdorferi*-positive during April, even though the spirochete was not detected in any of the larvae ($n = 180$) and unfed nymphs ($n = 155$) which fed upon these animals. During July and August, respectively, 14 of 21 and three of 21 mice captured within the maple forest had at least one *B. burgdorferi*-positive tissue (Table 3). *Borrelia burgdorferi* was isolated from 14 of 29 spleens, 13 of 29 bladders, eight of 29 ears

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

and five of 29 kidneys from infected mice collected on Long Point; it was recovered from more than one tissue in seven mice (Table 3).

At the maple forest, 42 (64%) of 66 captured mice were negative for *B. burgdorferi* infection by both culture and xenodiagnosis, while the organism was recovered by both of these techniques from eight of 66 mice. Interestingly, 15 (23%) of 66 mice had *B. burgdorferi*-positive organs but did not infect *I. scapularis* ticks; in contrast, only one mouse infected feeding *I. scapularis* larvae, yet yielded no *B. burgdorferi* on culture of four tissues; this mouse was captured in July. There was a poor to moderate level of agreement (κ = 0.35) between xenodiagnosis and bacterial culture for detecting *B. burgdorferi* infections in mice.

DISCUSSION

Regardless of the method of inoculation, most mice experimentally infected with *B. burgdorferi* were capable of infecting *I. scapularis* larvae only during the period beginning 7 DPI; transmission of *B. burgdorferi* to feeding ticks was minimal from mice at 21, 35 and 49 DPI. The duration of *B. burgdorferi* infectivity for feeding *I. scapularis* varies depending on the host species and route of inoculation. A greater proportion of field-collected white-footed mice were capable of infecting *I. scapularis* ticks (90%) than chipmunks (*Tamias striatus*) (75%) or meadow voles (*Microtus pennsylvanicus*) (6%) (Mather et al., 1989b). Rabbits (*Oryctolagus cuniculus*) inoculated subcutaneously with *B. burgdorferi* infected *I. scapularis* larvae from 12 to 15 DPI, but not from 4 to 9 or 16 to 18 DPI (Burgdorfer, 1984). White-tailed deer (*Odocoileus virginianus*) syringe-inoculated with *B. burgdorferi* were infectious to feeding *I. scapularis* larvae 43 DPI (Oliver et al., 1992), and the bacteria also was cultured from the ears of inoculated deer 10 wk PI (Luttrell et al., 1994). *Borrelia burgdorferi* was recovered from larvae that had fed on syringe-inoculated chipmunks 4 mo PI (McLean et al., 1993).

In our study, more *I. scapularis* larvae became infected when fed upon tick-infected mice than on syringe-inoculated mice. This is consistent with several previous studies (Donahue et al., 1987; Mather and Mather, 1990). In our study, the duration of *B. burgdorferi* infectivity was not influenced by method of inoculation. In contrast, mice (*Mus musculus*) inoculated by syringe with *B. burgdorferi* infected fewer *Ixodes ricinus* larvae than animals infected by tick feeding (Gern et al., 1993). Mode of inoculation (syringe vs. tick feeding) also influences the expression of clinical Lyme borreliosis in dogs (Appel et al., 1993).

In our study, the ability of both experimentally-inoculated and naturally-infected mice to transmit *B. burgdorferi* to feeding ticks did not always correlate with the presence of the bacteria in host tissues. Whether our animals with *B. burgdorferi*-infected tissues might have developed recrudescent spirochetemias at some later date, and then been capable of infecting feeding ticks, is unknown. The distinction between infected animals (culture positive) and infective animals (capable of infecting feeding ticks) should be made whenever possible. Clearly, studies on the infectivity of hosts, based primarily on xenodiagnostic techniques, should be run in parallel with agent identification in tissue (culture or polymerase chain reaction), to clarify the course and epidemiologic significance of *B. burgdorferi* infection in mice and other hosts.

On Long Point, habitat-specific differences were observed in the proportion of mice infective to feeding *I. scapularis* larvae. Similarly, the prevalence of *B. burgdorferi* infection, based on culture, within mice captured during the same month, varied among different habitats in Wisconsin (USA) (Callister et al., 1988); shrews (*Blarina brevicauda*) captured at three localities in Massachusetts (USA) also had varying capacity to infect ticks (Telford et

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

al., 1990). The low prevalence of *B. burgdorferi* infection in mice captured at the cottonwood dune habitat was not unexpected, since *I. scapularis* is very rare within this habitat (L. R. Lindsay, unpubl. data). However, the recovery of *B. burgdorferi* from the tissues of six of 62 animals from this habitat is evidence either that there is tick transmission even though tick densities are very low (Callister et al., 1991; Lord et al., 1994), or that contact or vertical transmission of *B. burgdorferi* is occurring (Burgess et al., 1986).

Within the maple forest, the proportion of *B. burgdorferi*-infected mice and recovery of *B. burgdorferi* by culture was greatest during July. Similarly, in Connecticut (USA), the greatest proportion of mice with *Borrelia burgdorferi*-positive organs was captured during June and July (Anderson et al., 1987). The proportion of bank voles (*Clethrionomys glareolus*) infecting *I. ricinus* larvae with *B. burgdorferi* in Sweden also varied during the year, with peak infectivity in August and September (Tälleklint et al., 1993). Recruitment in reservoir populations, at a time when nymphs are inactive, is likely responsible for declines in *B. burgdorferi* infectivity. On Long Point, the decline in *B. burgdorferi* infectivity of mice from July to August was unrelated to recruitment because the proportion of new individuals in the mouse population was similar in July (14%) and August (9%) (data not shown).

The proportion of our mice that infected xenodiagnostic ticks (14%), and the proportion of *I. scapularis* ticks infected by feeding on these animals (29%), were lower than the values reported from Massachusetts (Mather et al., 1989a, b), but are comparable to the results of studies from Connecticut (Anderson et al., 1983), Wisconsin (Callister et al., 1988) and Maine (USA) (Rand et al., 1993). The prevalence of *B. burgdorferi* infection in host-seeking *I. scapularis* nymphs collected within the maple forest (n = 461) varied from 17 to 35% annually between 1990 and 1993 (Lindsay et al., 1991; L. R. Lindsay, unpubl. data). The proportion of *I. scapularis* infected when larvae are fed upon infective mice from Long Point (Table 3) or on experimentally-infected mice (Table 1) approached these values. Locality-specific variation in tick density, relative densities of competent and essentially incompetent reservoir hosts (such as white-tailed deer), and age and strain differences in mice and *B. burgdorferi* among localities are likely responsible for some of the observed variability. Regional variability in *B. burgdorferi* infectivity of mice may also reflect fundamental differences in the cycle of *B. burgdorferi* infection at these various localities.

According to Spielman et al. (1985), the inverted pattern of seasonal activity of *I. scapularis*, where infected nymphs from the previous season feed prior to the larvae of the year, amplifies *B. burgdorferi* infection within mouse populations. Under this scenario, after exposure to infected *I. scapularis* nymphs from May to July, mice remain infective for several weeks or months, and transmit *B. burgdorferi* infection to the next cohort of *I. scapularis* larvae that emerges to seek hosts during August and September.

In our study, the duration of *B. burgdorferi* infectivity in experimentally-infected mice was short-lived (<3 wk) and apparently, the same was true of mice naturally-infected on Long Point. Even in the highly enzootic maple forest habitat, few mice were infected with *B. burgdorferi*, or infectious to experimentally-applied larvae, at the time of larval emergence (mid-August). Therefore, it is unlikely that many free-ranging *I. scapularis* larvae would acquire *B. burgdorferi* infection at this time. *Ixodes scapularis* larvae that overwinter unfed, and feed during June and July of the next year, would have a higher probability of acquiring *B. burgdorferi*, since a greater proportion of mice are infective at this time. Once infected, these fed larvae would molt into nymphs later the same year or in the following year; then they would transmit *B. burgdorferi* infection to

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

mice in May to July. Because of the apparently short duration of *B. burgdorferi* infectivity in mice, maintenance of the organism among mouse and tick populations on Long Point may require that *I. scapularis* larvae and nymphs share common hosts at approximately the same time of year; i.e., co-feeding (Gern and Rais, 1996; Randolph et al., 1996) occurs.

In this circumstance, environmental conditions that influence the size of overwintering larval and nymphal tick populations may be critical to the prevalence of *B. burgdorferi* in the tick population. Since in most years on Long Point, overwintering larval populations are large (L. R. Lindsay, unpubl. data) this may have mitigated against selection for strains of *B. burgdorferi* that are persistently infectious for ticks. This contrasts with localities where overwintering populations of larvae are small, in which case selection pressure for persistence of infectivity of mice for ticks would occur, to permit intergenerational transmission of *B. burgdorferi*. Tälleklint and Jaenson (1995) considered that diapausing *I. ricinus* are the main overwintering reservoir for *B. burgdorferi* in Sweden. On Long Point, the non-infectious status of overwintering mice which harbored *B. burgdorferi* in April similarly suggests that it is overwintering infected *I. scapularis* nymphs, rather than persistently infected vertebrate hosts, that are the reservoir of *B. burgdorferi* infection during the period of the year when immature ticks are inactive.

ACKNOWLEDGMENTS

Special thanks are due to J. Robinson, P. Ashley and D. Brown for providing access to the Long Point National Wildlife Area. Research assistance was provided by J. Heal, G. Jones, M. Coburn, W. Bennett, L. McGuffin, B. Sun, D. Bridle and J. Rombeek. A. Barbour graciously provided monoclonal antibodies. Logistical and technical requirements were supported by the Canadian Wildlife Service and the Ontario Ministry of Agriculture, Food and Rural Affairs. H. Artsob provided advice and encouragement. Financial support was provided by a Natural Science and Engineering Research Council of Canada postgraduate scholarship (L.R.L.) and by National Health Research and Development Grant #6606-4083-54 from Health and Welfare Canada (I.K.B., G.A.S. and S.A.M.).

LITERATURE CITED

ANDERSON, J. F. 1988. Mammalian and avian reservoirs for *Borrelia burgdorferi*. Annals of the New York Academy of Science 539: 180–191.

———, L. A. MAGNARELLI, W. BURGDORFER, AND A. G. BARBOUR. 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. American Journal of Tropical Medicine and Hygiene 32: 818–824.

———, R. C. JOHNSON, L. A. MAGNARELLI, AND F. W. HYDE. 1985. Identification of endemic foci of Lyme disease: Isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). Journal of Clinical Microbiology 22: 36–38.

———, R. C. JOHNSON, AND L. A. MAGNARELLI. 1987. Seasonal prevalence of *Borrelia burgdorferi* in natural populations of white-footed mice, *Peromyscus leucopus*. Journal of Clinical Microbiology 25: 1564–1566.

APPEL, M. J. G., S. ALLAN, R. H. JACOBSON, T. L. LAUDERDALE, Y. F. CHANG, S. J. SHIN, J. W. THOMFORD, R. J. TODHUNTER, AND B. A. SUMMERS. 1993. Experimental Lyme disease in dogs produces arthritis and persistent infection. Journal of Infectious Diseases 167: 651–664.

BARBOUR, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale Journal of Biology and Medicine 57: 521–525.

BARKER, I. K., G. A. SURGEONER, H. ARTSOB, S. A. MCEWEN, L. A. ELLIOTT, G. D. CAMPBELL, AND J. T. ROBINSON. 1992. Distribution of the Lyme disease vector, *Ixodes dammini* (Acari: Ixodidae) and isolation of *Borrelia burgdorferi* in Ontario, Canada. Journal of Medical Entomology 29: 1011–1022.

BARTHOLD, S. W., D. H. PERSING, A. L. ARMSTRONG, AND R. A. PEEPLES. 1993. Kinetics of *Borrelia burgdorferi* dissemination and evolution of disease after intradermal inoculation of mice. American Journal of Pathology 139: 263–273.

BATTALY, G. R., AND D. FISH. 1993. Relative importance of bird species as hosts for immature *Ixodes dammini* (Acari: Ixodidae) in a suburban residential landscape of southern New York. Journal of Medical Entomology 30: 740–747.

BURGDORFER, W. 1984. The New Zealand white rabbit: An experimental host for infecting ticks with Lyme disease spirochetes. Yale Journal of Biology and Medicine 57: 609–612.

BURGESS, E. C., T. E. AMUNDSON, J. P. DAVIS, R. A. KASLOW, AND R. EDELMAN. 1986. Experimental inoculation of *Peromyscus* spp. with *Borrelia*

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

burgdorferi: Evidence of contact transmission. American Journal of Tropical Medicine and Hygiene 35: 355–359.

CALLISTER, S. A., W. A. AGGER, R. F. SCHELL, AND J. L. E. ELLINGSON. 1988. *Borrelia burgdorferi* infection surrounding La Crosse, Wis. Journal of Clinical Microbiology 26: 2632–2636.

———, J. A. NELSON, R. F. SCHELL, D. A. JOBE, R. BAUTZ, W. A. AGGER, AND J. COGGINS. 1991. Survey for *Ixodes* spp., and *Borrelia burgdorferi* in southeastern Wisconsin and northeastern Illinois. Journal of Clinical Microbiology 29: 403–406.

DONAHUE, J. G., J. PIESMAN, AND A. SPIELMAN. 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. American Journal of Tropical Medicine and Hygiene 36: 92–96.

GERN, L., AND O. RAIS. 1996. Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acari: Ixodidae). Journal of Medical Entomology 33: 189–192.

———, U. E. SCHAIBLE, AND M. M. SIMON. 1993. Mode of inoculation of the Lyme disease agent *Borrelia burgdorferi* influences infection and immune responses in inbred strains of mice. The Journal of Infectious Diseases 167: 971–975.

GODSEY, M. S. JR., T. E. AMUNDSON, E. C. BURGESS, W. SCHELL, J. P. DAVIS, R. KASLOW, AND R. EDELMAN. 1987. Lyme disease ecology in Wisconsin: Distribution and host preferences of *Ixodes dammini*, and prevalence of antibody to *Borrelia burgdorferi* in small mammals. American Journal of Tropical Medicine and Hygiene 37: 180–187.

JOHNSON, S. E., G. C. KLEIN, G. P. SCHMID, G. S. BOWEN, J. C. FEELY, AND T. SCHULZE. 1984. Lyme disease: a selective medium for isolation of the suspect etiological agent, a spirochete. Journal of Clinical Microbiology 19: 81–82.

LEVINE, J. F., M. L. WILSON, AND A. SPIELMAN. 1985. Mice as reservoirs of the Lyme disease spirochete. American Journal of Tropical Medicine and Hygiene 34: 355–360.

LINDSAY, L. R., I. K. BARKER, G. A. SURGEONER, S. A. MCEWEN, L. A. ELLIOTT, AND J. KOLAR. 1991. Apparent incompetence of *Dermancentor variabilis* (Acari: Ixodidae) and fleas (Insecta: Siphonaptera) as vectors of *Borrelia burgdorferi* in an *Ixodes dammini* endemic area of Ontario, Canada. Journal of Medical Entomology 28: 750–753.

LORD, R. D., V. R. VERONICA, J. G. HUMPHREYS, AND R. G. MCLEAN. 1994. Distribution of *Borrelia burgdorferi* in host mice in Pennsylvania. Journal of Clinical Microbiology 32: 2501–2504.

LUTTRELL, M. P., K. NAKAGAKI, E. W. HOWERTH, D. E. STALLKNECHT, AND K. A. LEE. 1994. Experimental infection of *Borrelia burgdorferi* in white-tailed deer. Journal of Wildlife Diseases 30: 146–154.

MARTIN, S. W., A. H. MEEK, AND P. WILLEBERG. 1987. Veterinary epidemiology: Principles and methods. Iowa State University press, Ames, Iowa, 343 pp.

MATHER, T. N., S. R. TELFORD III, A. B. MACLACHLAN, AND A. SPIELMAN. 1989a. Incompetence of catbirds as reservoirs for the Lyme disease spirochete (*Borrelia burgdorferi*). The Journal of Parasitology 75: 66–69.

———, M. L. WILSON, S. I. MOORE, J. M. C. RIBEIRO, AND A. SPIELMAN. 1989b. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). American Journal of Epidemiology 130: 143–150.

———, AND M. E. MATHER. 1990. Intrinsic competence of three ixodid ticks (Acari) as vectors of the Lyme disease spirochete. Journal of Medical Entomology 27: 646–650.

MCLEAN, R. G., S. R. UBICO, AND L. M. COOKSEY. 1993. Experimental infection of the eastern chipmunk (*Tamias striatus*) with the Lyme disease spirochete (*Borrelia burgdorferi*). Journal of Wildlife Diseases 29: 527–532.

OLIVER, J. H. JR., D. STALLKNECHT, F. W. CHANDLER, A. M. JAMES, B. S. MCGUIRE, AND E. HOWERTH. 1992. Detection of *Borrelia burgdorferi* in laboratory-reared *Ixodes dammini* (Acari: Ixodidae) fed on experimentally inoculated white-tailed deer. Journal of Medical Entomology 29: 980–984.

———, M. R. OWSLEY, H. J. HUTCHESON, A. M. JAMES, C. CHEN, W. S. IRBY, E. M. DOTSON, AND D. K. MCLAIN. 1993. Conspecificity of the ticks *Ixodes scapularis* and *Ixodes dammini* (Acari: Ixodidae). Journal of Medical Entomology 30: 54–63.

RAND, P. W., E. H. LACOMBE, R. P. SMITH, JR., S. M. RICH, C. W. KILPATRICK, C. A. DRAGONI, AND D. CAPORALE. 1993. Competence of *Peromyscus maniculatus* (Rodentia: Cricetidae) as a reservoir host for *Borrelia burgdorferi* (Spirochaetares: Spirochetaceae) in the wild. Journal of Medical Entomology 30: 614–618.

RANDOLPH, S. E., L. GERN, AND P. A. NUTTALL. 1996. Co-feeding ticks: Epidemiological significance for tick-borne pathogen transmission. Parasitology Today 12: 472–479.

REZNICEK, A. A., AND P. M. CATLING. 1989. Flora of Long Point, Regional Municipality of Haldimand-Norfolk. Michigan Botanist 28: 99–175.

SAS INSTITUTE. 1988. SAS/STAT User's guide, Release 6.03 ed. SAS Institute, Cary NC.

SNEDECOR, G. W., AND W. G. COCHRAN. 1989. Statistical methods. Iowa State University Press, Ames, Iowa, 507 pp.

SPIELMAN, A., M. L. WILSON, J. F. LEVINE, AND J. PIESMAN. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annual Review of Entomology 30: 439–460.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TÄLLEKLINT, L., AND T. G. T. JAENSON. 1995. Is the small mammal (*Clethrionomys glareolous*) or the tick vector (*Ixodes ricinus*) the primary overwintering reservoir for the Lyme borreliosis spirochete in Sweden? Journal of Wildlife Diseases 31: 537–540.

———, ———, AND T. N. MATHER. 1993. Seasonal variation in the capacity of the bank vole to infect larval ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. Journal of Medical Entomology 30: 812–815.

TELFORD, S. R. III, T. N. MATHER, G. H. ADLER, AND A. SPIELMAN. 1990. Short-tailed shrews as reservoirs of the agents of Lyme disease and human babesiosis. The Journal of Parasitology 76: 681–683.

Received for publication 6 July 1994.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use