BORRELIA BURGDORFERI INFECTION IN WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS) IN HEMLOCK (TSUGA CANADENSIS) HABITAT IN WESTERN PENNSYLVANIA

Authors: Lord, Rexford D., Humphreys, Jan G., Lord, Veronica R., McLean, Robert G., and Garland, Curtis L.

Source: Journal of Wildlife Diseases, 28(3) : 364-368

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-28.3.364

Journal of Wildlife Diseases, 28(3), 1992, pp. 364–368

BORRELIA BURGDORFERI INFECTION IN WHITE-FOOTED MICE (*PEROMYSCUS LEUCOPUS*) IN HEMLOCK (*TSUGA CANADENSIS*) HABITAT IN WESTERN PENNSYLVANIA

Rexford D. Lord,[1] Jan G. Humphreys,[1] Veronica R. Lord,[2] Robert G. McLean,[3] and Curtis L. Garland[1]

[1] Department of Biology, Indiana University of Pennsylvania, Indiana, Pennsylvania 15705, USA
[2] Brucellosis Laboratory, Institute of Veterinary Medicine, 2102 Maracay, Venezuela
[3] Division of Vector-borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, U.S. Public Health Service, Department of Health and Human Services, Box 2087, Fort Collins, Colorado 80522, USA

ABSTRACT: White-footed mice (*Peromyscus leucopus*) were captured and their tissues sampled from 27 sites in seven counties of western Pennsylvania in 1990 for isolation and identification of *Borrelia burgdorferi*. Two hundred sixty mice were captured from which there were 27 isolations. Significantly more mice were captured and significantly more isolations made from hemlock (*Tsuga canadensis*) habitat than from deciduous species forest. Hemlock habitat is sparse and focal but evidently increases winter survival of mice, and thus possibly results in increased infection rates in mice.

Key words: *Borrelia burgdorferi*, white-footed mice, field sampling, hemlock, habitat prevalence, *Peromyscus leucopus*, *Tsuga canadensis*.

INTRODUCTION

The involvement of white-footed mice (*Peromyscus leucopus*) as reservoir hosts of the Lyme borreliosis agent *Borrelia burgdorferi* (Burgdorfer et al., 1982) has been amply demonstrated (Bosler et al., 1984; Levine et al., 1985; Anderson et al., 1987a, b; Donahue et al., 1987). The deer tick *Ixodes dammini* (Spielman et al., 1979) has been repeatedly confirmed to be the principal vector of the spirochete in the northeastern United States (Anderson et al., 1983; Bosler et al., 1983; Levine et al., 1985).

The importance of white-tailed deer (*Odocoileus virginianus*) in maintaining *I. dammini* tick populations and to the incidence of Lyme borreliosis is well documented (Main et al., 1981; Bosler et al., 1984; Magnarelli et al., 1984; Wilson et al., 1985). To a certain extent, the population density of white-footed mice and its role in the epizootiology of this disease is known in New England (Anderson et al., 1987a; Mather et al., 1989) but has received less attention elsewhere.

Lyme borreliosis has expanded westward from the New England states and New York and New Jersey during the last decade and has become established in Pennsylvania (Steere, 1989). The majority of the human cases reported are from the southeastern region of the state although cases have been reported from western counties (Brittingham, 1989). Little is known about the natural reservoir hosts or tick vectors in Pennsylvania.

A premise of this study was that the agent of Lyme borreliosis would be unevenly distributed throughout all the various ecosystems, and this would be demonstrable through differential infection rates of mammal hosts in different habitats. Another premise was that small mammals, always the most abundant, would prove useful indicators. Thus, the purpose of the study was to sample different habitats by trapping the small mammal species and to determine which species best revealed the habitat best suited for *B. burgdorferi*.

MATERIALS AND METHODS

To study the role of small mammal populations in the enzootiology of Lyme borreliosis required knowledge of an area already known to contain the disease. Reports of a higher than usual number of human cases in the city of St. Marys, Elk County (41°29'N, 78°31'W), in western Pennsylvania suggested the possible presence of an endemic focus in that area. Lack of precisely where in Elk County persons were

being infected led to an initial period of "prospecting" for *B. burgdorferi* in white-footed mice and other small mammals around the city of St. Marys.

A trap (30 × 7.6 × 7.6 cm, Sherman live trap) for small mammals was selected to sample any species up to a small squirrel in size. The bait selected (peanut butter) attracts all small rodents and shrews. Forty traps (wrapped in several layers of newspaper and supplied with cotton for protection against the cold) were set 18 February 1990 (Table 1) and recovered the following day. Ten traps were set at a distance of about 5 km each to the north, south, east and west of the city. Traps were set at 10-m intervals in a line and when possible were placed next to fallen tree trunks. They were placed in exactly the same pattern in all habitats (hemlock or deciduous forest).

Initial and subsequent findings guided later placement of traps in diverse habitats. Habitats sampled included hemlock clumps located in valleys along streams and softwood deciduous forest (aspen, red maple) also in valleys along streams. Hillside slopes and the top of the Allegheny Plateau were hardwood forests (oak, hickory) with well drained topography. On each occasion traps were set for one night only. At the end of the study, tracking boards (Lord, 1983) were used to independently determine *Peromyscus* sp. activity in the various habitats to confirm the trapping results.

Tissues (bladder and spleen) from mammals captured were aseptically removed, triturated in BSK medium (Barbour, 1984), then inoculated in tubes containing 7 ml of BSK and incubated at 34 C. Later samples from the tubes were examined using a darkfield microscope, at intervals of 2 to 4 days for a month. Spirochete positive samples were passed to other BSK tubes and slides were prepared for fluorescent antibody (FA) examination. A drop of culture was air dried on a slide, fixed in cold acetone for 10 min, dried and stored at 20 C. In preparation for FA examination, a drop of fluorescein-conjugated rabbit anti-*Borrelia burgdorferi* IgG (Centers for Disease Control, Fort Collins, Colorado 80522, USA) was placed on the dried preparation and incubated at 25 C in a moist, dark chamber for 30 min. The slide was shaken to remove conjugate, immersed in distilled water for 10 min, air dried, covered with a drop of 10% glycerine in water and a cover slip, and examined using a fluorescence microscope.

Bladder and ear tissues taken from mice captured in March (Table 1) were tested (Schwan et al., 1988), the results of which led to subsequent sampling of ears only (Sinsky and Piesman, 1989). Approximately one third of the ear from anesthetized mice was severed with scissors, dipped in 70% ethyl alcohol, placed in 10% liquid bleach for 10 min, then dipped in alcohol again and flamed before being placed in 0.05 ml of BSK media in a plastic tube. The sample was then triturated in the tube with a broken glass rod, 0.02 ml of the suspension passed to a tube with 7 ml of BSK, and processed as previously stated. Representative isolates were shipped to the CDC Laboratory (Fort Collins, Colorado) where they were confirmed as being *B. burgdorferi* by the indirect FA test using anti-OspA monoclonal antibodies H5322 (Barbour et al., 1986) with known strains of *B. burgdorferi* (B-31) and *B. hermsii* as positive and negative controls. The isolates were placed in the CDC *Borrelia* spp. reference collection (PA91-480 through PA91-488).

TABLE 1. Capture of white-footed mice and isolation of *Borrelia burgdorferi* spirochetes in western Pennsylvania, 1990.

Month	County	Mice capture/ Trap-nights	Isolates
February	Elk	11/40	1
March	Elk	27/60	11
April	Elk	19/80	1
June	Clearfield	6/48	
July	Elk	11/72	1
August	Elk	3/32	1
September	Elk	63/150	1
September	Westmoreland	18/60	
September	Cameron	11/40	
October	Elk	38/90	4
October	Jefferson	5/10	
November	Indiana	56/120	7
November	Somerset	6/20	
November	Cambria	3/10	
Total	7 counties	260/842	27

Ticks were captured by flagging or from mice as corollary to the small mammal studies. Ticks were identified, then dissected and suspensions of midgut examined using a darkfield microscope. Positive samples were confirmed by FA as described previously.

The Chi square and Fisher's Exact tests were used to statistically analyze the data.

RESULTS

Table 1 lists the months, counties, mice captured, traps set and isolates made. In the traps, white-footed mice prevailed numerically, but we also captured two red-backed voles (*Clethrionomys gapperi*), three southern flying squirrels (*Glaucomys volans*), three short-tailed shrews (*Blarina*

brevicauda), and three red squirrels (*Tamiasciurus hudsonicus*).

Two hundred sixty white-footed mice were captured in 842 trap-nights (one trap in the field for one night = 1 trap night). Twenty-seven sites in seven counties (Cameron, Clearfield, Elk, Indiana, Jefferson, Somerset, and Westmoreland) were sampled, with some sites in Elk and Indiana counties sampled repeatedly (Table 1).

Two markedly different habitats sampled were mixed deciduous (hardwoods and softwoods) forests and hemlock (*Tsuga canadensis*) with deciduous trees. Capture rates varied between these two habitats between seasons. In late winter and spring (February and March), 53 mice were taken in 140 trap-nights (38%) in hemlock, while only four mice were trapped in 40 trap-nights (10%) in deciduous forest; the difference was significant ($\chi^2 = 5.65$, $P = 0.02$). In fall (September and October) 58 mice were taken in 145 trap-nights (40%) in hemlock habitat, but in deciduous forest 77 mice were captured in 205 trap-nights (38%) (difference not significant, $\chi^2 = 0.04$).

To reconfirm the high trapping success in hemlock habitat, at the end of April, 10 traps were set (five each) in two hemlock sites west of St. Marys, and 10 traps were placed in deciduous forest between the two hemlock sites. Likewise 20 tracking boards were placed in the same pattern (traps and boards at 10-m intervals in a line) in both habitats. Five mice were captured in the hemlock habitat and two in the deciduous forest. In like manner, the tracking boards reflected the difference; six of 10 boards were positive for *Peromyscus* sp. in hemlock habitat, but only one of 10 boards was positive from the deciduous forest site.

Mice were trapped in valleys, hillsides and on top of the Allegheny Plateau. The capture rate in the valleys was 199 mice in 564 trap-nights (35%), on hillsides 43 mice in 193 trap-nights (22%), and on top of the plateau 18 mice in 85 trap-nights (21%). The effect of topography on the capture rate was significantly different ($\chi^2 = 3.14$, $P = 0.08$) for the capture rate between valleys and the plateau, and between valleys and hillsides ($\chi^2 = 5.65$, $P = 0.02$), but the difference between hillsides and plateaus was not significant ($\chi^2 = 0.00$).

Twenty-seven isolates of *B. burgdorferi* were made from white-footed mice during the entire study; 22 were made from 158 mice (14%) taken in hemlock habitat, while five isolates were made from 102 mice (5%) from deciduous forest and the difference was significant ($\chi^2 = 3.65$, $P = 0.05$). Isolates were from 24 of 199 mice (12%) taken in valleys, from one of 43 mice (2%) taken from hillsides, and from two of 18 mice (11%) taken from the top of the plateau. Analysis of the difference in the isolation rate showed no difference (OR = 0.91) between valleys and plateaus, but the difference between valleys and hillsides was significant (OR = 0.17, $P = 0.09$).

Five isolations of *B. burgdorferi* were made from *I. dammini* ticks; three were from adults and two were from nymphs (15% of 34 adults and nymphs). All isolations from ticks came from the region 5 km W of St. Marys, and all were from collections made by flagging in May. All of these isolations were from the valley with three from deciduous forest and two from hemlock habitat.

DISCUSSION

In late winter, more mice were captured in hemlock habitat than in deciduous forest habitat. However, by fall the capture rates in these two habitats were similar, indicating that summer reproduction in deciduous forest habitat compensated for the difference. Conversely, the winter survival of mice in the hemlock habitat appears to have been superior. The cones of hemlock provide a reliable source of seeds for food for the mice throughout the winter, and the trees provide cover from heat loss through radiation during cold nights, and reduce wind velocity, thus reducing the wind chill factor.

Isolation rates also were greater in the hemlock habitat than in the deciduous for-

est. Possibly the increased survival of mice in hemlock results in an increased availability of infected mice for larval and nymphal ticks during the spring and summer, increasing the transmission to other mice.

The 12 isolations made in late winter (February and March), plus seven isolations made in early winter (November) (Table 1), indicate the possibility of overwintering by *B. burgdorferi* in white-footed mice. Winters in the Elk County region are severe with little chance of tick activity before early May. Those isolations were made from mice captured with snow on frozen ground.

This study indicates the usefulness of detecting enzootic foci of *B. burgdorfer* through sampling white-footed mice. After finding a high prevalence of isolations in mice from hemlock habitat in Elk County, we later gave attention to hemlock habitat in Indiana County, a county with a low incidence of human cases. Positive results indicate the importance of hemlock habitat for aiding in directing future studies as well as possible selective control measures.

ACKNOWLEDGMENTS

We are thankful to the following for aid during the progress of this study: David Dennis, Michael Kesner, Alicia Linzey, Andrew Spielman and Ray Winstead. This project received financial support from the C.D.C., U.S. Public Health Service, Contract 200-90-0825. The University of Pittsburgh at Bradford loaned traps for this work.

LITERATURE CITED

ANDERSON, J. F., 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, AND L. A. MAGNARELLI. 1987a. Seasonal prevalence of Borrelia burgdorferi in natural populations of white-footed mice (*Peromyscus leucopus*). Journal of Clinical Microbiology 25: 1564–1566.

———, R. C. JOHNSON, L. A. MAGNARELLI, F. W. HYDE, AND J. E. MEYERS. 1987b. Prevalence of *Borrelia burgdorferi* and *Babesia microti* in mice on islands inhabited by white-tailed deer. Applied and Environmental Microbiology 53: 892–894.

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

———, S. F. HAYES, R. A. HEILAND, M. E. SCHRUMPF, AND S. L. TESSIURIER. 1986. A *Borrelia*-specific antibody binds to a flagellar epitope. Journal of Infection and Immunity 52: 549–554.

BOSLER, E. M., J. L. COLEMAN, J. L. BENACH, D. A. MASEY, J. P. HANRAHAN, W. BURGDORFERI, AND A. B. BARBOUR. 1983. Natural distribution of the *Ixodes dammini* spirochete. Science 220: 321–322.

———, B. G. ORIMSTON, J. L. COLEMAN, J. P. HANRAHAN, AND J. L. BENACH. 1984. Prevalence of the Lyme disease spirochete in populations of white-tailed deer and white-footed mice. Yale Journal of Biology and Medicine 57: 651–659.

BRITTINGHAM, M. C. 1989. Lyme disease. Extension circular 366. Pennsylvania State College of Agriculture Cooperative Extension, University Park, Pennsylvania, 5 pp.

BURGDORFER, W., A. G. BARBOUR, S. F. HAYES, J. L. BENACH, E. GRUNWALDT, AND J. P. DAVIS. 1982. Lyme disease—a tick-borne spirochaetosis? Science 216: 1317–1319.

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.

LEVINE, J. R., 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.

LORD, R. D. 1983. Rodent control programs: Use of the inked tracking board method in Mexico. Bulletin of the PanAmerican Health Organization 17: 259–268.

MAGNARELLI, L. A., J. F. ANDERSON, AND W. A. CHAPPELL. 1984. Antibodies to spirochetes in white-tailed deer and prevalence of infected ticks from foci of Lyme disease in Connecticut. Journal of Wildlife Diseases 20: 21–26.

MAIN, A. J., H. E. SPRANCE, K. O. KLOTER, AND S. E. BROWN. 1981. *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer (*Odocoileus virginianus*) in Connecticut. Journal of Medical Entomology 18: 487–492.

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

SCHWAN, T. G., W. BURGDORFER, AND C. F. GARCON. 1988. Changes in infectivity and plasmid profile

of the Lyme disease spirochete, *Borrelia burgdorferi* as a result of in vitro cultivation. Journal of Infection and Immunity 56: 1831–1836.

SINSKY, R. J., AND J. PIESMAN. 1989. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. Journal of Clinical Microbiology 27: 1723–1727.

SPIELMAN, A., C. M. CLIFFORD, J. PIESMAN, AND M. D. CORWIN. 1979. Human babesiosis on Nantucket Island, USA: Description of the vector, *Ixodes* (*Ixodes*) *dammini*, n. sp. (Acarina: Ixodidae). Journal of Medical Entomology 15: 218–234.

SPURR, S. H. 1964. Forest ecology. Ronald Press Company, New York, New York, 352 pp.

STEERE, A. C. 1989. Medical progress: Lyme disease. New England Journal of Medicine 321: 586–596.

WILSON, M. L., G. H. ADLER, AND A. SPIELMAN. 1985. Correlation between abundance of deer and that of the deer tick, *Ixodes dammini* (Acari: Ixodidae). Annals of the Entomological Society of America 78: 171–176.

Received for publication 9 July 1991.