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Experimental Infection of the Red-backed Vole (*Clethrionomys gapperi*) with *Borrelia burgdorferi*

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ABSTRACT: Red-backed voles (*Clethrionomys gapperi*) were live trapped in northern St. Louis County, Minnesota (USA), in late September and October 1988 and experimentally inoculated with *Borrelia burgdorferi*. Spirochetes were isolated from most animals 14 and 28 days following inoculation. Thus, red-backed voles exposed to *B. burgdorferi* were susceptible to infection and could be a reservoir host, along with chipmunks (*Tamias striatus*) and other small rodents, in areas where white-footed mouse (*Peromyscus leucopus*) populations are low. No evidence of clinical disease was noted in any infected voles.

Key words: *Borrelia burgdorferi*, Lyme disease, red-backed vole, *Clethrionomys gapperi*, experimental infection.

Lyme disease, a tick transmitted infection with the spirochete, *Borrelia burgdorferi*, is enzootic in several areas of the United States, Europe and Eurasia (Branton et al., 1992; Marin Canica et al., 1993; Fukunaga et al., 1993). The primary vector of this disease in the upper midwestern United States, particularly in Minnesota and Wisconsin, is *Ixodes scapularis* (Oliver et al., 1993), a three host tick (Anderson and Magnarelli, 1984). While the tick has been found on a wide variety of mammalian and avian hosts, the immature stages commonly feed on small rodents, particularly the white-footed mouse (*Peromyscus leucopus*) and chipmunks (*Tamias striatus*) (Callister et al., 1988; Godsey et al., 1987; Mather et al., 1989). The preferred host of adult ticks is the white-tailed deer (*Odocoileus virginianus*) (Wilson et al., 1986). In Minnesota, *Borrelia burgdorferi* could readily be isolated from *Peromyscus* spp. coming from areas where Lyme disease is enzootic, while no isolation was made from red-backed voles (*Clethrionomys gapperi*) collected from the same areas (Loken et al., 1985). In Europe, the bank vole (*Clethrionomys glareous*) is considered a reservoir for this spirochete (Hovmark et al., 1988). Red-backed voles frequently are the predominant rodent in coniferous and mixed forests in northern Minnesota (Hazard, 1982). Since areas in which the red-backed vole predominates include areas where Lyme disease is not prevalent, it is possible that the composition of the small mammal population may be a significant factor in determining the prevalence of the disease, either due to varying susceptibilities of different species to infection or their suitability as a host for the tick vector. Our objective was to evaluate the susceptibility of the red-backed vole to experimental infection with *B. burgdorferi*.

Red-backed voles were live-trapped (Havahart, Lititz, Pennsylvania, USA) in an area free of *I. scapularis* and Lyme disease in northern St. Louis County, Minnesota (92°22′W longitude, 47°42′N latitude), in late September and October 1988 (Loken et al., 1985). The area was believed to be free of ticks based on surveys taken during the falls of 1982 through 1989 (Minnesota Department of Health, 1990). Further evidence of the *I. scapularis*-free nature of the area was obtained by a tick survey conducted with the cooperation of hunters who submitted ticks from their dogs (Drew et al., 1988). In addition, 85 red-backed voles were snap-trapped in the area, examined for the presence of ticks, and cultured for *B. burgdorferi* as described by Loken et al. (1985). Traps were set, baited with peanut butter and checked every 30 to 60 min. All animals examined were found free of ticks and spirochetes. Twenty-eight live-trapped voles were kept in captivity for 10 mo prior to inoculation. Voles were removed from live-traps, placed in wire topped polycarbonate cages with

cedar shavings. Rodent chow, seeds, fresh grapes and water were provided ad libitum. Trapped voles were transported to the laboratory within 48 hr of collection. One person was assigned to their total care. Bedding was changed weekly with food and water provided ad libitum. Over time the voles became semidomesticated and were not afraid of this particular individual.

Borrelia burgdorferi, strain MM1, initially isolated from the kidney of a *Peromyscus* sp. (Loken et al., 1985) was used in this study. The first subculture of this organism had been maintained in liquid nitrogen. Frozen material was thawed, inoculated into 8.0 ml tubes of Barbour-Stoenner-Kelly II (BSKII), and incubated at 30 C for 7 days (Barbour, 1984). The cell number was determined by the use of a Petroff-Hausser counting chamber (VWR Scientific, Greenbelt, Maryland, USA) and adjusted to a density of 7×10^6 spirochetes per ml. Two groups of 10 voles and two groups of 10 non-Swiss Albino mice (Hsd: NSA [CF1] Harlan-Sprague Dawley, Inc., Indianapolis, Indiana, USA) were inoculated intraperitoneally with 1.0 ml of spirochete suspension. A group of 10 mice and eight voles remained as control animals. Ten mice and 10 voles were killed at 2 wk and another 10 mice and 10 voles were killed at 4 wk following inoculation. Samples of liver (two lobes), spleen, and kidneys were aseptically removed from control and infected animals, macerated through a sterile 3 cc syringe into BSKII medium. Serial ten-fold dilutions, through 1:10,000 were prepared in BSKII medium and incubated as described by Barbour (1984). The identify of the isolated spirochetes was confirmed with known monoclonal antibody against Osp A, a 31,000 kDa protein (Barbour et al., 1983). Tissue samples of kidneys, spleen, liver, lungs and brain, from control and infected animals, were fixed in 10% neutral buffered formalin and embedded in paraffin; 5 μm tissue sections were stained with hematoxylin and eosin and Warthin-Starry silver stain for spirochetes (Thompson, 1966).

Borrelia burgdorferi was isolated from eight of 10 red-backed voles 14 days after inoculation and from six of 10 voles after 28 days. Spirochetes were isolated from all laboratory mice at both 14 and 28 days following needle inoculation. *Borrelia burgdorferi* was isolated most frequently from the kidneys of both species. Isolations were made from the kidneys of 12, the spleens of six, and the livers of two voles. Isolations were made from the kidneys of 14, the spleens of six and the livers of four CF1 mice. No evidence of clinical disease (lameness) or gross lesions was observed in any of the animals. On microscopic examination of embedded tissues, we observed slight to moderate lymphoid hyperplasia in the white pulp of the spleens stained with hematoxylin and eosin. No lesions were observed in the other tissues examined nor were spirochetes observed in the silver stained tissue sections, except for control animals' spleens ($n = 3$) and kidneys ($n = 8$) which were positive. All uninoculated voles and CF1 mice remained negative throughout the experiment as determined by organ culture and microscopic examination of silver stained tissues.

The geographical distribution of Lyme disease in northeastern and northcentral United States has been correlated with the distribution of *I. scapularis* (Steere and Malawista, 1979; Spielman et al., 1985) and the white-tailed deer (Wilson et al., 1986). Nymphal ticks were more prevalent in areas where white-tailed deer were abundant (Duffy et al., 1994), providing evidence that deer are required for the development of high tick concentrations. However, these ticks are absent from large areas of northern Minnesota where deer and other suitable reservoir hosts also are prevalent (Loken et al., 1985; Drew et al., 1988). Since the range of *I. scapularis* is reported to be expanding (Anderson et al., 1988), it is possible that the tick may even-

tually spread to these areas (Anderson et al., 1988; Schultz et al., 1986). Recovery of immature *I. scapularis* on a variety of avian hosts is evidence that migrating birds, which move through areas where ticks are abundant, should have disseminated them throughout a larger area (such as Connecticut (USA) (Anderson and Magnarelli, 1984) or Wisconsin (McLean et al., 1993b; Weisbrod and Johnson, 1989)). It appears that factors other than a suitable deer population and a mode of tick movement are necessary to develop and maintain *I. scapularis* populations. One important factor required for the development and maintenance of tick populations is a suitable environment with appropriate relative humidities which allow for the development of tick larval and nymph stages (Platt et al., 1992; Stafford, 1994). In addition, severe winters with little snow cover, or extreme cold, may lower the overwintering success of *I. scapularis* in this area.

Red-backed voles are a predominant rodent in many areas of northern Minnesota. Immature *I. scapularis* have been found on red-backed voles in the United States; thus, there is a potential for natural exposure to the etiologic agent of Lyme disease (Carey et al., 1980; Rand et al., 1993). Based on our results, spirochetes can be recovered from the tissues of voles for at least 28 days following experimental inoculation with a relatively high dose of organisms. It is not known if voles are able to become infected with a lower dose of organisms, but probably depends on the minimum number of organisms needed for infection to occur. Isolations from experimentally inoculated voles compared favorably with the results reported by others who inoculated *Peromyscus* spp. (Donahue et al., 1987; Wright and Nielsen, 1990) and chipmunks (McLean et al., 1993b). The presence of spirochetes in the tissues is evidence the red-backed vole could possibly serve as a reservoir for *B. burgdorferi* from which ticks could become infected. However, for the red-backed vole to serve as a reservoir, spirochetes would have to be present in sufficient numbers and duration to facilitate the infection of ticks feeding on these animals (Telford et al., 1988). At the present time it is not known if this could occur and thus could be a limiting factor in the establishment of this disease in a given area.

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