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Isolation of *Borrelia burgdorferi* from *Peromyscus leucopus* in Oklahoma

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ABSTRACT: *Borrelia burgdorferi* was isolated from a field-caught *Peromyscus leucopus* from central Oklahoma (USA). The strain was identified as *B. burgdorferi* by reaction with monoclonal antibody H5332 specific for the outer surface protein OspA of *B. burgdorferi*. This represents the first isolation of *B. burgdorferi* from a wild mouse outside of the normal range of the known vectors *Ixodes dammini* and *I. pacificus*.

Key words: *Borrelia burgdorferi*, Lyme Disease, *Peromyscus leucopus*, Oklahoma.

Lyme disease is a zoonotic tick-borne spirochetosis caused by the bacterium *Borrelia burgdorferi*. The disease is the most commonly reported human tick-borne illness in the United States (Centers for Disease Control, 1991; Ciesielski et al., 1988) and is the only tick-borne spirochete thus far isolated in the eastern United States (Anderson et al., 1989). Although Lyme disease has been reported from 46 states, the vast majority of cases are recorded in several northeastern, upper midwestern and Pacific northwestern states (Centers for Disease Control, 1991). Although variations in case definitions appear to exist among states, increased numbers of reports have occurred since the late 1980's in the lower midwestern and southern states (Anderson et al., 1989; Miller et al., 1990). Confirmed human Lyme disease cases in Oklahoma between 1988 and 1990 are 8, 25, and 13 respectively (Reiner et al., 1991).

Most information on the epidemiology, ecology, and transmission of *B. burgdorferi* in the U.S. comes from areas where *I. dammini* and/or *I. pacificus* are endemic. Information on vectors, transmission, and wildlife reservoirs from non-endemic locations of the U.S. is scanty. Although transmission studies have not been accomplished, Teltow et al. (1991) report spirochetes in several arthropods from non-endemic areas. Isolation and transmission of the infective agent are essential for determining vectors and wildlife reservoirs in geographic locations outside of the highly endemic areas of the U.S. As part of an ongoing evaluation of ecological factors influencing the occurrence and transmission of *B. burgdorferi* in Oklahoma, we attempted to isolate *B. burgdorferi* from wild-caught rodents from central Oklahoma.

The study was conducted over a 20-month-period (1989 to 1991) in five counties (Lincoln, Noble, Osage, Pawnee, and Payne) of central Oklahoma (USA) (35°50′N to 36°50′N, 97°00′W to 98°00′W). Wild-caught mice and rats were collected using Sherman live-traps (H. B. Sherman Traps Inc., Tallahassee, Florida, USA) and Tomahawk double door live-traps (National Live Trap Company, Tomahawk, Wisconsin, USA) set weekly and baited with a combination of peanut butter and oatmeal. All live rodents were returned to the laboratory and killed with chloroform. No attempt was made to determine ectoparasite infestations. Liver, spleen, kidney, heart and urinary bladder were removed aseptically under a sterile hood and placed separately into sterile mortars. Approximately 2 ml of sterile BSK II media (Barbour, 1984) was added and the tissue aseptically ground with a pestle. A 100 μl portion of media containing ground tissue was inoculated into tubes with 6.5 ml BSK II media, and incubated at 33 to 34 C. Cultures were examined weekly for 6 wk by dark-field microscopy for the presence of spirochetes. A 0.5 ml portion of cultures

showing spirochetes was inoculated subcutaneously into each of six tick-free laboratory reared *P. leucopus*. Mice were killed after 6 wk for spirochete re-isolation in BSK II media following the same procedures.

Fifty-three mice (*P. leucopus*) and 52 rats (51 *Sigmodon hispidus*, and 1 *Neotoma floridana*) were trapped. Spirochetes were isolated from the urinary bladder of one adult male *P. leucopus* from Payne County. Based on an ocular micrometer, organisms ranged in length from 8.8 to 12 μm during the first 3 wk of culture; longer forms (18 to 28 μm) were observed during subsequent culture. Spirochetes (PlOK) were re-isolated from all six laboratory-reared *P. leucopus* in BSK II media using the precedures described above, and are being maintained in laboratory-reared *P. leucopus* and in BSK II media.

Whole cells of the isolated spirochetes were solubulized at 100 C for 10 min, in sodium dodecyl sulfate (SDS) sample buffer (2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue). Proteins were separated by SDS-PAGE (polyacrylamide gel electrophoresis) on a 0.75 mm thick, 10% separating gel (29:1, acrylamide : bis) using a discontinuous buffer system (Laemmli, 1970). Proteins were transferred to nitrocellulose (Duralose UV, Stratagene Cloning Systems, La Jolla, California, USA) using the Mini-Transblot apparatus (Bio-Rad, Inc., Richmond, California, USA) for 15 min at 15V, 15 min at 25V, 15 min at 40V, 20 min at 50V, and 30 min at 75V. After blocking with 1% gelatin (Bio-Rad Inc.), blots were incubated with either the *B. burgdorferi*-specific, anti-OspA monoclonal antibody (MAb) H5332 (Barbour et al., 1983) or the *Borrelia* genus-specific anti-41 kDa flagellar antigen MAb H9724 (Barbour et al., 1986). MAb-antigen interactions were detected with affinity purified, goat anti-mouse alkaline phosphatase conjugate (Gibco BRL, Inc., Gaithersburg, Maryland, USA).

Based on the SDS-PAGE analysis of polypeptides, we observed a major protein

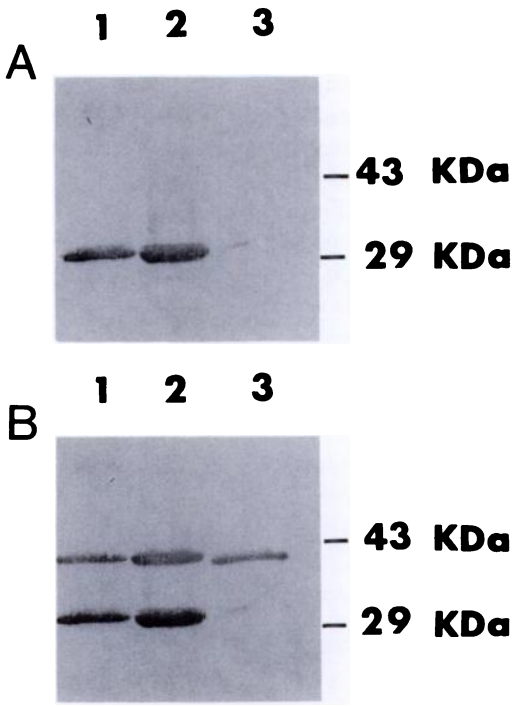

FIGURE 1. Immunoblot analysis of total cellular proteins form (1) Oklahoma mouse isolate (PLOK), (2) *B. burgdorferi* JDI, and (3) *B. hermsii*. Panel A is the blot after probing with H5332, the *B. burgdorferi*-specific, anti-OspA MAb. Panel B is the same blot reprobed with H9724, the *Borrelia* genus-specific MAb, which recognizes a 41 kDa flagellar antigen.

of PlOK at 31 kDa (data not shown). The *B. burgdorferi*-specific MAb H5332 reacted strongly in a Western blot with the 31 kDa protein of PlOK and with a protein of similar size in *B. burgdorferi* (JDI) whole cell lysates (Fig. 1) but did not react with *Leptospira* spp. whole cell antigens (data not shown) nor with *B. hermsii* whole cell antigens (Fig. 1). MAb H9724 reacted with a similar sized antigen (41 KO_2) in PlOK, *B. burgdorferi* (JDI) and *B. hermsii* (Fig. 1).

This report is the first confirmed isolation of *B. burgdorferi* from *P. leucopus* at a site where both *I. dammini* and *I. pacificus* are absent. Although this information does not confirm the role of *P. leucopus* in the transmission of *B. burgdorferi* to humans in Oklahoma, it does document *P. leucopus* as a possible natural reservoir

for this organism in this region of the country. No efforts were made to determine if the rodents were infested with ticks, but rodents from the same areas are infested with larval and nymphal *Dermacentor variabilis*, *Ixodes scapularis*, and larval *Amblyomma americanum* (A. A. Kocan, unpubl. data). Because of the wide range of potential tick vectors that could feed on infected mice, we do not speculate on a natural tick vector in Oklahoma.

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