EXPERIMENTAL BORRELIA BURGDORFERI INFECTIONS IN THE WHITE-FOOTED MOUSE, DEER MOUSE, AND FULVOUS HARVEST MOUSE DETECTED BY NEEDLE ASPIRATION OF SPIROCHETES

Authors: NieLin, Ge, and Kocan, A. Alan

Source: Journal of Wildlife Diseases, 29(2) : 214-218

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-29.2.214

Journal of Wildlife Diseases, 29(2), 1993, pp. 214–218

EXPERIMENTAL *BORRELIA BURGDORFERI* INFECTIONS IN THE WHITE-FOOTED MOUSE, DEER MOUSE, AND FULVOUS HARVEST MOUSE DETECTED BY NEEDLE ASPIRATION OF SPIROCHETES

Ge NieLin and A. Alan Kocan

Department of Veterinary Parasitology, Microbiology and Public Health,
College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma 74078, USA

ABSTRACT: Three methods were tested for recovering *Borrelia burgdorferi* from live mice onto BSK II culture medium. Four laboratory-reared *Peromyscus leucopus* were inoculated intraperitoneally with the JD-1 isolate of *Borrelia burgdorferi*. *Borrelia burgdorferi* spirochetes were recovered from 13 of 20 (65%) samples taken by needle aspiration between days 7 and 40 post-inoculation (PI) and from 1 of 16 samples of skin obtained by ear punch biopsy during the same sampling period. Spirochetes were not recovered from culture media inoculated with mouse blood. The use of needle aspirates for recovering spirochetes was compared among three species of mice: *P. leucopus*, *P. maniculatus*, and *Reithrodontomys fulvescens*. Spirochetes were isolated from 14 of 15 aspiration samples from four *P. maniculatus*, 12 of 20 from three *P. leucopus*, and 15 of 20 from four *R. fulvescens* taken between days 7 and 48 PI. Spirochetes were isolated from only one aspiration sample between days 80 and 95 PI from any of the mice tested. Needle aspiration was an efficient method for repeated recovery of *B. burgdorferi* from live, experimentally infected mice. We also document *R. fulvescens* as an experimental host for *B. burgdorferi*. Based on their susceptibility to infection, all species of mice tested herein may play a role in the epidemiology of Lyme disease where their distribution is compatable with endemic transmission.

Key words: Lyme disease, recovery, needle aspiration, skin punch biopsy, blood, *Borrelia burgdorferi*, *Peromyscus leucopus*, *Peromyscus maniculatus*, *Reithrodontomys fulvescens*.

INTRODUCTION

Lyme disease is a tick-borne spirochetosis with a worldwide distribution (Burgdorfer et al., 1982; Johnson et al., 1984a; Steere et al., 1983). The etiologic agent, *Borrelia burgdorferi* (Burgdorfer et al., 1982; Johnson et al., 1984b), is transmitted by ticks of the *Ixodes ricinus* complex (Steere et al., 1983). The white-footed mouse (*Peromyscus leucopus*) is an important reservoir for *B. burgdorferi* (Levine et al., 1985; Donahue et al., 1987), being susceptible to infection under both natural and experimental conditions. This species has been widely used in xenodiagnosis and tick transmission studies (Anderson et al., 1985; Donahue et al., 1987; Wright and Nielsen, 1990). In addition to *P. leucopus*, several species of wild mice and rats are susceptible to natural or experimental infection with *B. burgdorferi* (Anderson et al., 1986; Burgess et al., 1986; Burgdorfer and Gage, 1987; Mather et al., 1989). In living mice, rats, rabbits, and hamsters, the spirochete has been isolated from blood, urine, ear tissue (Bosler and Schulze, 1986; Sinsky and Piesman, 1989; Barthold et al., 1991; Moody and Barthold, 1991), and needle aspirates of skin (Piesman et al., 1991). At necropsy, spirochetes have been isolated from spleen, kidney, liver, blood, urinary bladder, brain and eyes (Bosler et al., 1983; Anderson et al., 1987; Schwan et al., 1988; Callister et al., 1989; Burgess et al., 1990; Wright and Nielsen, 1990), with the urinary bladder being the tissue from which organisms were most consistently isolated (Schwan et al., 1988).

The objectives of this study were to compare methods of repeated recovery of *B. burgdorferi* from live *P. leucopus*. Once determined, the most efficient method on recovery of spirochetes was employed on three species of mice to determine their susceptibility to infection, as well as the length of time during which spirochetes could be recovered by repeated sampling of the live mice.

MATERIALS AND METHODS

Peromyscus leucopus used in this study were obtained as a closed outbred colony, four to seven generations in captivity. Breeding mice had been maintained as a closed, tick-free colony in a facility separate from experimentally inoculated animals. Based on routine culture in BSK II media (Barbour, 1986) of liver, kidney, spleen, heart, and urinary bladder from mice in this colony over the past 3 yr, the mice were free of infection with *B. burgdorferi*. Deer mice (*P. maniculatus*) and fulvous harvest mice (*Reithrodontomys fulvescens*) were obtained from colonies at the Department of Entomology, Oklahoma State University, Oklahoma (USA), and were maintained as a closed colony, one to two generations from the wild. Different species of mice were housed under tick-free conditions. Food and water were provided ad libitum.

The spirochete inoculum (*B. burgdorferi* strain JD1) was a first passage reisolate from experimentally infected *P. leucopus*. This strain had been maintained in BSK II media and by mouse-medium passage, and was identified as *B. burgdorferi* using monoclonal antibody H5332 (Barbour et al., 1983; Kocan et al., 1992). This isolate was chosen because it was infective for *P. leucopus* (Kocan et al., 1992) and at the time of this study, isolates had not been obtained from Oklahoma (Mukolwe et al., 1992). Prior to use, each new batch of BSK II medium was tested by inoculation with several recognized strains of *B. burgdorferi* (Kocan et al., 1992) and used for isolation attempts only if it was capable of maintaining spirochetes.

Recovery of *B. burgdorferi* from live, experimentally infected *P. leucopus*

Four *P. leucopus* were inoculated intraperitoneally with 0.5 ml of BSK II medium containing approximately 5×10^6 spirochetes. Two uninoculated mice from the same colony were used as controls.

Blood samples were taken from the tail vein and skin samples were taken by ear punch biopsy (Sinsky and Piesman, 1989) on days 7, 15, 23, and 31 post-inoculation (PI). Needle aspirates were collected on days 7, 15, 23, 31, 40, 84, and 118 PI according to the procedures of Piesman et al. (1991); however, we used the thighs of the mouse instead of the ears. The posterior thighs of each mouse were shaved with a surgical blade and cleaned with 70% ethanol. One ml of BSK II medium was injected intradermally with a 3-ml syringe and 21-g needle. The fluid was simultaneously aspirated with another needle placed adjacent to the inoculation site. Approximately 0.3 to 0.6 ml of fluid was recovered using this procedure.

TABLE 1. Recovery of *Borrelia burgdorferi* from whole blood, skin taken by ear punch biopsy, and needle aspiration in live experimentally-infected *Peromyscus leucopus*.

Days post-inoculation	Method of recovery		
	Blood	Ear punch biopsy	Needle aspiration
7	0/4[a]	0/4	2/4
15	0/4	0/4	1/4
23	0/4	1/4	3/4
31	0/4	0/4	3/4
40	ND[b]	ND	4/4
84	ND	ND	0/4
118	ND	ND	0/4

[a] Number positive/number examined.
[b] Not done.

At 150 days PI, all mice were killed, and heart, liver, spleen, kidney and urinary bladder were placed into BSK II media as previously described (Mukolwe et al., 1992). A small piece of bladder tissue was placed directly into the medium while spleen, kidney and liver were ground in a sterile mortar prior to placing 100 µl of each ground suspension into the medium. Cultures were incubated at 34 C and examined weekly for 4 to 6 wk by dark-field microscopy for the presence of spirochetes.

Recovery of spirochetes by needle aspiration from three different species of experimentally infected mice

Four *P. leucopus*, three *P. maniculatus* and four *R. fulvescens* were inoculated subcutaneously with *B. burgdorferi* and samples were taken by needle aspiration as described previously, on days 7, 12, 24, 34, 48, 80, and 95 PI. Two uninoculated mice of each species served as controls.

RESULTS

Spirochetes were recovered from 13 of 20 samples from experimentally infected *P. leucopus* taken by needle aspiration between 7 and 40 days PI (Table 1). However, spirochetes were not recovered from cultures taken at 84 and 118 days PI. Spirochetes were not detected in cultures inoculated with whole blood obtained 7 to 31 days PI and in only one of the 16 cultures inoculated with tissue taken by skin punch biopsy during this same time period (Table 1).

At necropsy, 150 days PI, spirochetes

TABLE 2. Recovery of *Borrelia burgdorferi* by needle aspiration from three species of live mice.

Days post-inoculation	Rodent species		
	Peromyscus leucopus	*Peromyscus maniculatus*	*Reithrodontomys fulvescens*
7	2/3[a]	3/3	3/4
12	1/3	3/3	3/4
24	2/3	2/3	2/4
34	3/4	3/3	4/4
48	4/4	3/3	3/4
80	0/4	1/3	0/4
95	0/4	0/3	0/4

[a] Number positive/number examined.

were obtained from two of four urinary bladder samples and one of four spleen samples from the *P. leucopus*. Spirochetes were not observed from cultures of heart, liver or kidney tissues. Spirochetes were not recovered by any of the sampling methods used or from any tissues from control mice.

In the second study, where spirochete recovery by needle aspiration was compared in the three species of laboratory-reared wild mice, *B. burgdorferi* was successfully isolated by needle aspiration from all species (Table 2). Spirochetes were cultured from needle aspirates from 14 of 15 *P. maniculatus*, 12 of 20 *P. leucopus* and 15 of 20 *R. fulvescens* taken between 7 and 48 days PI. After 80 days PI, spirochetes were isolated from only a single *P. maniculatus* while no spirochetes were obtained after this time from either *P. leucopus* or *R. fulvescens*. At necropsy 150 days PI, spirochetes were isolated in cultures inoculated with urinary bladder tissue of two of four *R. fulvescens* but not from any tissues from *P. leucopus* or *P. maniculatus*. Spirochetes were not isolated from control mice at any sample time from needle aspirates or tissues obtained at necropsy.

DISCUSSION

Isolation of *B. burgdorferi* by needle aspiration from animal skin was described by Piesman et al., (1991), as a method for identifying infections in live experimentally infected rabbits. In their study, using BSK II media as an aspirate, nine of 12 samples were positive for spirochetes. In the present study, spirochetes were most consistently recovered from live experimentally infected *P. leucopus* by needle aspiration of media when compared with attempted recovery from blood or ear punch biopsy samples. Needle aspiration also was effective for recovering spirochetes from experimentally infected live *P. maniculatus* and *R. fulvescens* between 1 and 7 wk PI. However, after 45 days PI, only one culture from any of the three species was positive. Thus, length of time in which *B. burgdorferi* persists in mouse skin appears to vary among animal species. Johnson et al., (1984a, 1988) demonstrated that *B. burgdorferi* can persist in hamster skin for several months. However, following tick transmission studies, Donahue et al. (1987) suggested that spirochete numbers gradually decrease in skin. In our study, using three species of mice, it appeared that spirochetes may be absent or too few in number to be detected by media cultivation after 80 days PI. By comparison, recovery of spirochetes at necropsy 150 days PI from the same mice demonstrated that *B. burgdorferi* persisted in the urinary bladder and spleen for a longer period than in skin.

Although ear punch biopsy has been shown to be a useful method of isolating spirochetes from some live animals (Sinsky and Piesman, 1989; Moody and Barthold, 1991), we were able to isolate spirochetes from only one of 16 such samples from experimentally infected *P. leucopus*. Barthold et al. (1991) reported similar results using this method to recover spirochetes from C3H mice sampled between 1 and 21 days PI. In addition, repeated sampling from the same mouse requires the removal of considerable ear tissue, thus limiting the number of times the animal can be sampled.

We were unable to isolate spirochetes from cultures inoculated with blood from

the same *P. leucopus* taken at 7 to 31 days PI. Burgess et al. (1986) isolated spirochetes from blood from one of 10 *P. maniculatus* 21 days PI. Barthold et al. (1991) isolated *B. burgdorferi* from the blood of experimentally infected C3H mice with greater success. However, Johnson et al. (1984a) obtained only approximately 40% positive cultures from blood obtained daily from five live Syrian hamsters on days 1 through 6, respectively, but was unable to recover spirochetes from blood taken after 1 wk PI. Similar results were obtained by Goodman et al. (1991), recovering spirochetes from two of four and one of five blood samples on days 5 and 7 PI, respectively, while not isolating spirochetes after that time. Wright and Nielsen (1990) recovered spirochetes on day 3 PI from one of 61 cultures inoculated with blood from *P. leucopus*. Additionally, spirochetemia in some infected animals, as indicated by successful cultivation, may last from 1 to 4 wk (Benach et al., 1984; Johnson et al., 1984a; Burgdorfer and Gage, 1987). Burgdorfer and Schwan (1991) demonstrated that a prolonged spirochetemia detected by xenodiagnosis using *I. dammini* may persist in *P. leucopus* for ≤3 mo. Burgdorfer and Gage (1987) demonstrated that spirochetemia in cotton rats (*Sigmodon hispidus*) was characterized by alternating spirochete-positive and negative periods similar to that seen in infections with the relapsing-fever spirochete, *B. hermsii*. Thus, the detection of spirochetes in cultures inoculated with whole blood may vary among animal species and sampling time.

Based on the present study, *B. burgdorferi* spirochetes persist in the skin of experimentally infected *P. leucopus, P. maniculatus* and *R. fulvescens* ≤7 wk after inoculation, and can be successfully recovered by repeated sampling from live mice using needle aspiration and culture medium. It also appears that, in addition to *P. leucopus* and *P. maniculatus, R. fulvescens* can serve as an experimental reservoir for *B. burgdorferi* spirochetes. Therefore, *R. fulvescens* may play a role in the epidemiology of this organism where distribution and ecological conditions would allow for natural *B. burgdorferi* infection and infestation by a suitable tick vector.

ACKNOWLEDGMENT

This study was supported in part by the Oklahoma Center for the Advancement of Science and Technology (OCAST) Project No. HR9-012 and published as Oklahoma State University, College of Veterinary Medicine publication No. 92-003. Technical assistance by Raymond Zinn and Lisa Narus is appreciated. We also thank Dr. Robert W. Barker, Department of Entomology for providing the *P. maniculatus* and *R. fulvescens* and Dr. Katherine M. Kocan for critical review of the manuscript.

LITERATURE CITED

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

———, R. C. JOHNSON, L. A. MAGNARELLI, AND F. W. HYDE. 1986. Culturing *Borrelia burgdorferi* from spleen and kidney tissues of wild-caught white-footed mice, *Peromyscus leucopus*. Zentralblatt für Bakteriologie, Microbiologie und Hygiene 263: 34–39.

———, ———, AND ———. 1987. Seasonal prevalence of *Borrelia burgdorferi* in natural populations of white-footed mice (*Peromyscus leucopus*). Journal of Clinical Microbiology 25: 1564–1566.

BARBOUR, A. G. 1986. Isolation and cultivation of Lyme disease spirochetes. Yale Journal of Biology and Medicine 57: 521–525.

———, S. L. TESSIER, AND W. J. TODD. 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by monoclonal antibody. Infection and Immunity 41: 795–804.

BARTHOLD, S. W., D. H. PERSING, A. L. AMSTRONG, AND R. A. PEEPLES. 1991. Kinetics of *Borrelia burgdorferi* dissemination and evolution of disease after intradermal inoculation of mice. American Journal of Pathology 139: 263–273.

BENACH, J. L., E. M. BOSLER, J. L. COLEMAN, AND G. S. HABICHE. 1984. Experimental transmission of the Lyme disease spirochete to rabbits. Journal of Infectious Diseases 150: 786–787.

BOSLER, E. M., AND T. L. SCHULZE. 1986. The prevalence and significance of *Borrelia burgdorferi* in the urine of feral reservoir hosts. Zen-

tralblatt für Bakteriologie, Microbiologie und Hygiene 263: 40–44.

———, J. L. COLEMAN, J. H. BENACH, D. A. MASSEY, J. P. HANRAHAN, W. BURGDORFER, AND A. G. BARBOUR. 1983. Natural distribution of the *Ixodes dammini* spirochete. Science 220: 321–322.

BURGDORFER, W., AND K. L. GAGE. 1987. Susceptibility of the hispid cotton rat (*Sigmodon hispidus*), to the Lyme disease spirochete. American Journal of Tropical Medicine and Hygiene 37: 624–628.

———, AND T. G. SCHWAN. 1991. Lyme borreliosis: A relapsing fever-like disease? Scandinavian Journal of Infectious Diseases 77: 17–22.

———, A. G. BARBOUR, S. F. HAYSE, J. E. BENCH, E. GRUNNOLDT, AND J. P. DAVIS. 1982. Lyme disease—A tick-borne spirochetosis? Science 216: 1317–1319.

BURGESS, E. C., T. E. AMUNDSON, J. P. DAVIS, R. A. KASLOW, AND R. EDELMAN. 1986. Experimental inoculation of *Peromyscus* spp. with *Borrelia burgdorferi*: Evidence of contact transmission. American Journal of Tropical Medicine and Hygiene. 35: 355–359.

———, J. B. FRENCH JR., AND A. GENDRON-FITZPATRICK. 1990. Systemic disease in *Peromyscus leucopus* associated with *Borrelia burgdorferi* infection. American Journal of Tropical Medicine and Hygiene 42: 254–259.

CALLISTER, S. M., W. A. AGGER, K. F. SCHELL, AND K. M. BRAND. 1989. Efficacy of urinary bladder for isolation of *Borrelia burgdorferi* from naturally infected, wild *Peromyscus leucopus*. Journal of Clinical Microbiology 27: 773–774.

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.

GOODMAN, J. L., P. JURKOVICH, C. KODNER, AND R. C. JOHNSON. 1991. Persistent cardiac and urinary tract infections with *Borrelia burgdorferi* in experimentally infected Syrian hamster. Journal of Clinical Microbiology 29: 894–896.

JOHNSON, R. C., N. MAREK, AND C. KODNER. 1984a. Infection of Syrian hamsters with Lyme disease spirochetes. Journal of Clinical Microbiology 20: 1099–1101.

———, G. P. SCHMID, F. W. HYDE, A. G. STEIGERWALT, AND J. BRENNER. 1984b. *Borrelia burgdorferi* sp. nov. etiologic agent of Lyme disease. International Journal of Systematic Bacteriology 34: 496–497.

———, C. KODNER, M. RUSSELL, AND P. H. DURAY. 1988. Experimental infection of the hamster with *Borrelia burgdorferi*. Annals of New York Academy of Sciences 539: 258–263.

KOCAN, A. A., S. W. MUKOLWE, G. L. MURPHY, R. W. BARKER, AND K. M. KOCAN. 1992. Isolation of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) from *Ixodes scapularis* (Acari: Ixodidae) and *Dermacentor albipictus* (Acari: Ixodidae) ticks from Oklahoma. Journal of Medical Entomology 29: 630–633.

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.

MATHER, T. N., M. L. WILSON, S. I. MOORE, J. M. RIBIRO, 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.

MOODY, K. D., AND S. W. BARTHOLD. 1991. Relative infectivity of *Borrelia burgdorferi* in Lewis rats by various routes of inoculation. American Journal of Tropical Medicine and Hygiene 44: 135–139.

MUKOLWE, S. W., A. A. KOCAN, R. W. BARKER, AND G. L. MURPHY. 1992. Isolation of *Borrelia burgdorferi* from *Peromyscus leucopus* in Oklahoma. Journal of Wildlife Diseases 28: 281–283.

PIESMAN, J., G. D. MAUPIN, E. G. CAMPOS, AND C. M. HAPP. 1991. Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi*, with description of a needle aspiration isolation method. Journal of Infectious Diseases 163: 895–897.

SCHWAN, T. G., W. BURGDORFER, M. E. SCHRUMPF, AND R. H. KARSTENS. 1988. The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (*Peromyscus leucopus*). Journal of Clinical Microbiology 26: 893–895.

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.

STEERE, A. C., R. L. GRODZICHI, A. N. KORNBLATT, J. E. CRAFT, A. G. BARBOUR, W. BURGDORFER, G. P. SCHMID, E. JOHNSON, AND S. E. MALAWISTA. 1983. The spirochetal etiology of Lyme disease. The New England Journal of Medicine 308: 733–740.

WRIGHT, S. D., AND S. W. NIELSEN. 1990. Experimental infection of the white-footed mouse with *Borrelia burgdorferi*. American Journal of Veterinary Research 51: 1980–1987.

Received for publication 13 March 1992.