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The Urinary Bladder, a Consistent Source of *Borrelia burgdorferi* in Experimentally Infected White-Footed Mice (*Peromyscus leucopus*)

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White-footed mice, *Peromyscus leucopus*, were experimentally infected in the laboratory with *Borrelia burgdorferi*, the causative agent of Lyme disease. After mice were infected by intraperitoneal or subcutaneous inoculation or by tick bite, attempts were made to culture spirochetes from the urinary bladder, spleen, kidney, blood, and urine. Spirochetes were most frequently isolated from the bladder (94%), followed by the kidney (75%), spleen (61%), and blood (13%). No spirochetes were isolated from the urine. Tissue sectioning and immunofluorescence staining of the urinary bladder demonstrated spirochetes within the bladder wall. The results demonstrate that cultivation of the urinary bladder is very effective at isolating *B. burgdorferi* from experimentally infected white-footed mice and that culturing this organ may be productive when surveying wild rodents for infection with this spirochete.

The white-footed mouse, *Peromyscus leucopus*, is not only one of the preferred hosts of larval and nymphal *Ixodes dammini*, but also the primary host for infecting this tick with *Borrelia burgdorferi*, the causative agent of Lyme disease (8, 10, 12, 15–18, 20). In our laboratory, this species of rodent is capable of infecting *I. dammini* for at least 65 days following infection by tick bite or parenteral inoculation (W. Burgdorfer, unpublished observations). Numerous isolations from tissues of wild white-footed mice have demonstrated the significance of these animals as potential sources of infection of this important human pathogen (1–6, 13). Not all tissues from an infected rodent, however, are likely to produce an isolate when cultured. Of the tissues tested, the most consistent sources of *B. burgdorferi* have been the spleen and kidneys, whereas the blood and other tissues have been less productive (4, 14).

White-footed mice have been successfully colonized at the Rocky Mountain Laboratories to provide hosts for experimental infection of ticks with *B. burgdorferi*, as well as for evaluating the infectiousness of spirochetal strains cultured from ticks, wild rodents, and humans suffering from Lyme disease. For this purpose, cultured spirochetes are inoculated into white-footed mice, and subsequently, we attempt to culture the spirochete from several tissues, including those of the urinary bladder. In fact, cultivation of spirochetes from the bladder has proven to be the most useful method in assessing infection. In this paper, we demonstrate the efficacy of isolating *B. burgdorferi* from the bladder compared with other tissues and suggest that this organ should be cultured during attempts to isolate the spirochete from naturally infected wild rodents.

MATERIALS AND METHODS

Isolates of *B. burgdorferi*. Isolate Sh-2-82 originated from naturally infected *I. dammini* collected on Shelter Island, New York, in 1982. Midgut suspensions of ticks were examined by dark-field microscopy, and those found infected with spirochetes were stored at −70°C in modified Kelly medium (7, 16) containing 30% glycerol. Later, inocula were prepared by triturating the rapidly thawed midgut tissues of two infected ticks in 2.0 ml of modified Kelly medium; 0.25 ml of this suspension was inoculated intramuscularly into a white-footed mouse. *I. dammini* larvae from an uninfected laboratory colony were infected by allowing them to feed on the spirochetemic mouse. These ticks were reared to adult stage, and one infected female was triturated and cultured in modified Kelly medium. Spirochetes of this culture, passaged 10 times or less, were used to infect white-footed mice. Inoculations with this isolate having 15 or more passages in culture did not infect white-footed mice and provided negative-control mice for the study.

Isolate CA-2-87 originated from a pool of eight adult *I. pacificus* collected by flagging in Tulare County, California, in 1987. Ticks were triturated, and spirochetes in the initial culture of modified Kelly medium were inoculated into white-footed mice.

Isolate ECM-NY-86 originated from a skin biopsy from an erythema chronicum migrans lesion of a human patient in New York in 1986. The original isolation was made in modified Kelly medium and was subsequently passaged five times in modified Kelly medium before freezing it at −70°C and later inoculating it into white-footed mice.

Inoculation of white-footed mice and isolation of spirochetes. White-footed mice were from an uninfected laboratory colony at Rocky Mountain Laboratories established from progeny of specimens obtained from J. Benach and E. Bosler, Department of Pathobiology, State of New York Department of Health, and State University of New York at Stony Brook. Adult male and female white-footed mice were each inoculated intraperitoneally (i.p.) or subcutaneously with approximately 3×10^7 to 7.5×10^7 *B. burgdorferi* removed from modified Kelly medium by centrifugation (9,000 × *g* for 15 min) and suspended in 0.5 ml of phosphate-buffered saline–5 mM $MgCl_2$ (pH 7.4). Numbers of spirochetes in cultures were determined by the counting method of Stoenner (21). Three mice were infected by allowing infected *I. dammini* nymphs to feed on them for 4 to 5 days. White-footed mice were caged separately to prevent contact transmission (11). At 2 to 47 weeks postinoculation, the mice were anesthetized, bled by cardiac puncture, and killed with ether, and their abdominal cavity was opened. If the urinary bladder contained urine, the urine was collected in a tuber-

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culin syringe with a 27-gauge needle and inoculated into 9 ml of modified Kelly medium. The bladder, spleen, and left kidney were removed, and each was triturated in 1 ml of modified Kelly medium in a glass tissue grinder. Two portions of approximately 0.5 ml of each suspension were inoculated into two tubes containing 9 ml of modified Kelly medium, one tube containing the antibiotics phosphomycin (100 μg/ml) and rifampin (50 μg/ml). Whole blood (0.1 ml) was also inoculated into modified Kelly medium with or without antibiotics. Culture tubes were incubated at 34°C and examined by dark-field microscopy every 2 to 4 days for 1 month. The presence of spirochetes in culture confirmed infection of the tissues tested. Twelve white-footed mice, inoculated i.p. with noninfectious (higher-passaged) spirochetes, were processed in the same manner and provided negative controls.

Tissue sectioning and microscopic examination. Before four urinary bladders were triturated, they were each cut into three pieces for culture, scanning electron microscopy, and tissue sectioning and fluorescent-antibody staining. The pieces for culture were triturated and put into modified Kelly medium as already described. The pieces for scanning electron microscopy were fixed in glutaraldehyde, attached to a stub with double-stick tape, critical point dried, coated with a 15-nm layer of gold-paladium alloy, and viewed with a scanning electron microscope (35-CF, JEOL, Ltd.). The pieces for sectioning were fixed in 10% Formalin and processed by standard techniques (19). Briefly, the fixed bladder tissues were dehydrated, cleared, and infiltrated with 80 to 100% ethanol, xylene, and paraffin, consecutively, for 16 h with an Autotechnicon. The tissues were embedded in paraplast, and serial 5-μm sections were mounted on glass slides. The sections were deparaffinated with xylene, rehydrated, stained with rabbit polyclonal antibody to *B. burgdorferi* conjugated with fluorescein isothiocyanate, and examined by fluorescence microscopy. One urinary bladder from an uninfected white-footed mouse was cultured, sectioned, and stained for a negative control.

RESULTS

B. burgdorferi was isolated from the bladder, kidney, spleen, and blood of white-footed mice at 2 and 3 weeks after i.p. inoculation. Initially, more isolations were made from the bladder and spleen; therefore, later attempts to isolate spirochetes from the kidney were not done. Subsequent attempts to isolate the spirochete from the spleen, however, were also less successful. For all tissues tested, spirochetes were most frequently isolated from the bladder (94%), followed by the kidney (75%) and the spleen (61%) (Table 1). Spirochetes were also isolated from 2 of 15 (13%) blood samples but not from any of 15 urine samples. No spirochetes were observed in five urine samples examined by dark-field microscopy. Because of our concern that i.p. inoculation might make the bladder more susceptible to infection, white-footed mice were also inoculated subcutaneously and infected by the bite of infected ticks; both routes of infection resulted in isolations of *B. burgdorferi* from bladders. One mouse that was infected 47 weeks previously by tick bite had an infected bladder that appeared necrotic by gross observation, having a thick, opaque, yellow wall. However, no histological examination of this specimen was done to determine if pathological changes were present. All the other bladders appeared normal, although most were infected.

Culture tubes inoculated with triturated bladder tissues all became positive with spirochetes by dark-field examination sooner than those containing other tissues, usually within 4 to 6 days. Cultures of infected spleen and kidney tissues usually took 8 to 20 days before spirochetes were detected.

Examination by scanning electron microscopy failed to demonstrate spirochetes associated with either the outer or the inner epithelial surface of bladders that were positive by culture. However, 5-μm sections of bladder tissues stained by fluorescent antibody demonstrated spirochetes within the bladder wall. In most cases, spirochetes were observed as single organisms in various locations, although foci of approximately 10 spirochetes were occasionally seen (Fig. 1). No spirochetes or anything similar to their appearance were seen in the stained sections of the bladder that was negative in culture.

DISCUSSION

White-footed mice are highly susceptible to *B. burgdorferi* and are of primary importance in the epidemiology of Lyme disease in the northern midwest and northeastern United States (1–6, 8, 12, 13, 16–18). Our results suggest that the urinary bladder may be as good as or better than the spleen and kidney as a source of *B. burgdorferi* in naturally infected wild populations of white-footed mice. These organs have been chosen because laboratory studies with Syrian hamsters resulted in *B. burgdorferi* being most frequently isolated from the spleen (62%) at 2 weeks after i.p. inoculation with 10^8 organisms (14). In our study, 61% of the spleens were positive when cultured, a result that is nearly identical to that reported for hamsters. However, the urinary bladders from 94% of experimentally infected white-footed mice were positive in culture. Bladders were infected following i.p., subcutaneous, or tick bite inoculation, and spirochetes could be cultured from this organ at 2 to 47 weeks postinoculation. Our results suggest that persistent infection of the bladder may occur in naturally infected white-footed mice and that *B. burgdorferi* may be cultivated from this organ more successfully than from others.

Urinary bladders were infected in white-footed mice from which we were unable to isolate spirochetes from either the blood or urine. Johnson et al. (14) were unable to culture spirochetes from the urine of infected Syrian hamsters. Bosler and Schulze (9), on the other hand, found *B. burgdorferi* in the urine of 50% of white-footed mice captured for the first time on Shelter Island, New York. The relatively high prevalence of borreliae in the urine, however, may have

TABLE 1. Isolation of *B. burgdorferi* from various organs and blood of *P. leucopus* experimentally infected by i.p. or subcutaneous inoculation or by tick bite

Isolate	Route	No. of mice	No. of wk p.i.[b]	No. of isolations[a]			
				Bladder	Spleen	Kidney	Blood
Sh-2-82	i.p.	6	2	6	6	6	2
		6	3	6	5	3	0
		1	5	1	–	–	0
		2	16	2	1	–	0
		4	2	4	1	–	–
CA-2-87	i.p.	4	4	4	2	–	–
ECM-NY-86	i.p.	2	2	2	1	–	–
CA-2-87	s.c.[c]	3	3	3	0	–	–
Sh-2-82	Bite	3	47	1	3	–	–

[a] –, Not tested. Percentages of isolations from the bladder, spleen, kidney, and blood were 94, 61, 75, and 13%, respectively.
[b] p.i., Postinoculation.
[c] s.c., Subcutaneous.

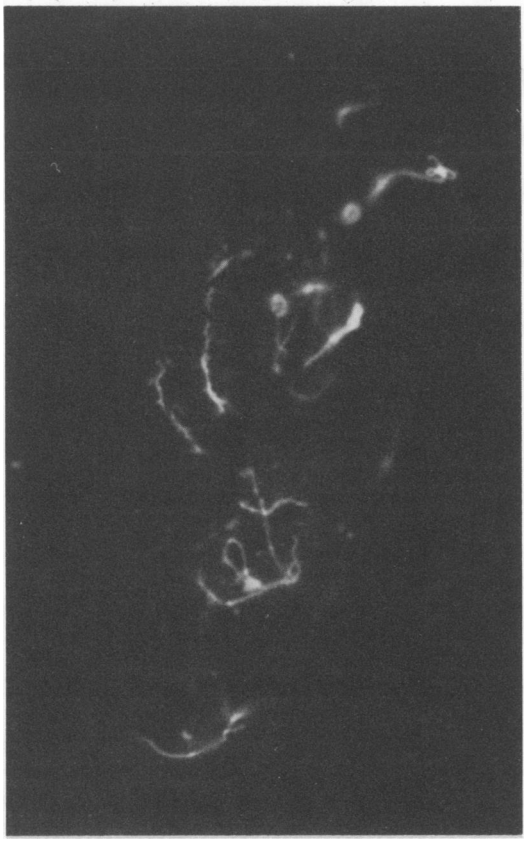

FIG. 1. The Lyme disease spirochete, *B. burgdorferi*, in the urinary bladder tissues of an experimentally infected white-footed mouse, stained by direct fluorescent antibody (magnification, ×2,800).

been influenced by concomitant infection with *Babesia microti*, which infected 95% of the spirocheturic mice.

The high percentage of bladder infections and the relative speed at which their cultures became positive indicate that *B. burgdorferi* may have a tropism for the urinary bladder. As there is yet no evidence that *B. burgdorferi* is an intracellular parasite, spirochetes in the bladder were most likely in the interstitial spaces. The potential pathogenic effect of the Lyme disease spirochete on the urinary bladder in these mice, as suggested by the one mouse infected for 47 weeks, requires further investigation. Our study demonstrates, however, that cultivation of the urinary bladder is very effective at isolating *B. burgdorferi* from experimentally infected white-footed mice and that culturing this organ may be of value when surveying wild rodents for infection with this spirochete.

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