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Efficacy of the Urinary Bladder for Isolation of *Borrelia burgdorferi* from Naturally Infected, Wild *Peromyscus leucopus*

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The efficacy of culturing urinary bladder tissue for *Borrelia burgdorferi* from naturally infected, wild *Peromyscus leucopus* mice was determined. The urinary bladder cultures were as efficient as spleen, kidney, and blood tissue cultures. The rapid *B. burgdorferi* isolation (mean, 6 days) from mouse urinary bladders should aid in defining new Lyme disease foci.

Infection with *Borrelia burgdorferi*, the etiologic agent of Lyme disease (5), has become increasingly recognized throughout the United States (9). Recent investigations have indicated that ticks of the genus *Ixodes* are the primary vector of *B. burgdorferi* in North America. These ticks will disperse to contiguous and noncontiguous areas if proper hosts are present (6, 8).

In endemic areas, the white-footed mouse, *Peromyscus leucopus*, has been shown to be an important *B. burgdorferi* reservoir (2, 3, 7, 8, 10, 12), and tissue cultures from white-footed mice have also proved useful for investigating potential Lyme disease foci (1, 2, 6, 11). *B. burgdorferi* has been most consistently isolated from the spleen and kidneys of infected *P. leucopus* mice.

Schwan et al. (12) recently demonstrated that the urinary bladders are more effective than other tissue cultures for isolating *B. burgdorferi* from experimentally infected white-footed mice. Mice were infected intraperitoneally, subcutaneously, or by tick bite. *B. burgdorferi* organisms were isolated from the bladder (94%), kidney (75%), and spleen (61%). Since these results were obtained from laboratory-infected mice, we investigated the efficacy of urinary bladder cultures for the isolation of *B. burgdorferi* from naturally infected, wild *P. leucopus* mice.

A total of 54 *P. leucopus* mice were captured from 31 May to 3 June 1988 at a known Lyme disease focus near Galesville, Wis. (6, 8). Mice were sacrificed within 24 h after capture. Forty (74%) mice were parasitized by *Ixodes dammini*. The mean numbers of larval and nymphal *I. dammini* per mouse were 2.1 and 1.0, respectively.

The tissues of the mice were cultured as described previously (2). Briefly, the kidneys, spleen, and bladder were removed aseptically and plunged through a needleless, 1-cm^3 tuberculin syringe into 6 ml of Barbour-Stoenner-Kelly medium (4, 8). Approximately 100 μl of blood was also obtained by cardiac puncture by using a tuberculin syringe with a 27-gauge needle and inoculated into Barbour-Stoenner-Kelly medium. In addition, urine present in the bladder was removed and cultured as described above.

Cultures were incubated at 31°C and examined by dark-field microscopy every 48 h for 22 days. Spirochetes were identified as *B. burgdorferi* by indirect fluorescence using monoclonal antibody H5332 (Alan Barbour, University of Texas, San Antonio), which reacts with a 31,000-kilodalton protein.

B. burgdorferi organisms were isolated from 23 (43%) of the 54 captured *P. leucopus* (Table 1). Isolates were obtained from 13 (57%) bladders, 13 (57%) spleens, 11 (48%) left kidneys, 9 (39%) right kidneys, and 9 (39%) blood cultures. No *B. burgdorferi* organisms were recovered from urine. When we compared the isolation rates using different combinations of tissue and blood cultures, no combinations resulted in significantly better *B. burgdorferi* isolation.

We found that *B. burgdorferi* organisms were detected from mouse urinary bladders in a mean of 6 days (standard error, 0.5 days), while the remaining tissues required 9 to 12 days of incubation before spirochetes were detected (Table 1). These results support the findings of Schwan et al. (12).

Schwan et al. (12) demonstrated that the bladder cultures from experimentally infected mice were more efficient (94%) than the spleen, kidney, and blood cultures for *B. burgdorferi* isolation. In contrast, we showed that the *B. burgdorferi* isolation rate from bladders of naturally infected mice was less efficient (57%) than from experimentally infected mice (12). One explanation may be the different methods used to process urinary bladders. Schwan et al. (12) triturated bladders, whereas we forced bladders through a syringe. In addition, splenic cultures were equally effective for *B. burgdorferi* isolation from naturally infected mice.

Although increased *B. burgdorferi* infection of urinary bladder tissue has been experimentally demonstrated by Schwan et al. (12), their animal model required a large *B. burgdorferi* inoculum, approximately 3×10^7 to 7.5×10^7 organisms. By contrast, the infective inoculum received naturally by mice is probably lower. When Schwan et al. (12)

TABLE 1. Isolation and recovery time of *B. burgdorferi* from tissues and blood of 23 wild, naturally infected *P. leucopus* mice

Sample	No. (%) of isolates[a]	No. of incubation days required for detection[b]
Bladder	13 (57)	6 ± 0.5
Blood	9 (39)	9 ± 0.4
Spleen	13 (57)	10 ± 1.0
Left kidney	11 (48)	11 ± 1.0
Right kidney	9 (39)	12 ± 2.0

[a] Number and percentage of *B. burgdorferi*-infected samples from 23 *P. leucopus* mice.
[b] Mean ± standard error ($n \geq 10$).

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infected mice with *I. dammini* feedings for 4 or 5 days, *B. burgdorferi* were isolated from only one (33%) bladder and three (100%) spleens. It is also likely that naturally infected mice are infected for different lengths of time. *B. burgdorferi* recovery from tissues of these mice may be influenced by the duration of infection.

We demonstrated that urinary bladder cultures were as efficient as cultures of the spleen and other tissues for *B. burgdorferi* isolation from naturally infected mice. The rapid *B. burgdorferi* isolation from mouse urinary bladders should aid in the detection of *B. burgdorferi* from endemic areas and help define new Lyme disease foci.

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