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The Isolation of *Borrelia burgdorferi* from Infected Laboratory Mice

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Lyme borreliosis is a zoonosis afflicting both humans and domestic animals as well as a wide range of feral animals including a variety of small rodents, rabbits, raccoons, skunks, deer, and ground-feeding birds (Anderson et al., 1983; Anderson et al., 1985; Anderson and Magnerelli, 1984; Bosler et al., 1983; Godsey et al., 1987; Fish and Daniels, 1990; Simpson and Hinck, 1993). In the northeastern United States, where it was discovered and remains highly endemic, the epidemiology is well understood. In the southern states, cases in humans are increasingly reported, yet the vectors, reservoir hosts, and epidemiologic patterns remain less clear (Burgdorfer and Gage, 1987; Cielsielski et al., 1988).

One method commonly employed for studying the distribution in feral hosts has been the cultivation of the etiologic agent, *Borrelia burgdorferi*, from spleen, kidneys and urinary bladder (Anderson et al., 1985; Schwann et al., 1988). Schwann et al. (1988) suggested that cultivation from the urinary bladder might be the most appropriate method for sampling feral rodents since laboratory studies with white mice revealed a 94% rate of infection for that organ. Both live traps and snap traps have been employed for procuring feral animals but definitive data have not been presented to compare the two methods with regard to success rate for cultivation from tissue samples. A preliminary study conducted in our laboratory indicated that the Lyme agent could be cultivated from tissues of laboratory infected white-footed mice (*Peromyscus leucopus*) when held for as long as 8 hours post-mortem at varying temperatures.

The current study was undertaken to better define the conditions of temperature and time post-mortem that would permit realistic expectations for cultivation from trapped rodents. Laboratory white mice were injected intraperitoneally with 0.3 mL of a suspension of *Borrelia burgdorferi*, strain SH-2-82, donated by Dr. Tom Schwann. The spirochetes had been permitted to grow in BSK-II medium (Barbour, 1984; Barbour, 1986; Berger et al., 1985) until a dense growth was observed by dark field microscopy. The mice were divided into five groups with 15 mice per group. Each group was subdivided into three subgroups of five animals per group to be necropsied at zero time, 4 hours, and 8 hours post-mortem. Holding temperatures for the 4-hr and 8-hr groups ranged from 5° C to 25° C. At 4 weeks post-inoculation, the animals were sacrificed by cervical dislocation. To minimize microbial surface contamination, they were denuded with a depilatory, treated with 5.25% hypochlorite for 3 minutes followed by a sterile distilled water rinse, and finally painted with a tincture of iodine. The spleen and urinary bladder of each were aseptically removed and triturated together in 2-mL of BSK-II medium with a sterile Pyrex glass tissue homogenizer. A 1 mL sample of the extract was inoculated into a sterile screw-capped tube containing 6 mL of BSK-II medium. The tubes were incubated at 34° C and examined at weekly intervals for growth by dark field microscopy. Tubes with no growth at 4 weeks were declared negative.

Comparisons with zero time controls (Table 1) indicate that cultivation attempts were equally successful in deceased animals held as long as 8 hours post-mortem at temperatures up to 15° C. At 20° C the recovery rates were reduced to 40% and 20% for the 4-hr and 8-hr groups respectively while the zero time controls provided a rate of 80%. At 25° C no recoveries were recorded at either post-mortem holding time, with the controls producing an 80% recovery once again.

Table 1. Cultivation of *Borrelia burgdorferi* from the spleen and urinary bladder of infected laboratory white mice.

Recovery Rates at Different Holding Times				
Holding Temperature (Celsius)	Hours:	0	4	8
5		4/5	3/5	4/5
10		3/5	4/5	3/5
15		4/5	3/5	4/5
20		4/5	2/5	1/5
25		4/5	0/5	0/5

The data suggest that snap traps can be utilized with a high probability of providing isolation of *B. burgdorferi* from tissues of infected feral animals when ambient temperatures are fairly cool. The use of snap traps during warmer months would require frequent monitoring so that deceased animals could be removed and necropsied at once. Animals taken during these periods would likely yield better results if live-trapping were employed.

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