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Isolation of *Borrelia burgdorferi* from the Blood of a Bushy-tailed Wood Rat in California

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ABSTRACT: *Borrelia burgdorferi* was isolated from the blood of a bushy-tailed wood rat (*Neotoma cinerea*) from northeastern California, USA. This is the first isolation of live *B. burgdorferi* spirochetes from a wild animal in California. Spirochetes were observed in stained tissues from the brain, liver, spleen, and kidneys.

Key words: *Borrelia burgdorferi*, Lyme disease, bushy-tailed wood rat, *Neotoma cinerea*.

Borrelia burgdorferi is the causative agent of Lyme disease. Attempts to isolate *B. burgdorferi* from the blood of wild mammals in California have been unsuccessful (Lane and Brown, 1991; Gordus, 1992). This paper documents the first isolation of *B. burgdorferi* from the blood of a bushy-tailed wood rat (*Neotoma cinerea*) collected from northeastern California.

In March 1987, one bushy-tailed wood rat was trapped in a Tomahawk live trap (Tomahawk, Wisconsin, USA) at "Hotel Rock" on Tule Lake National Wildlife Refuge (NWR), Siskiyou County, California, USA (41°50′N, 121°30′W). Five cc of whole blood was collected by cardiac puncture with a 6 cc syringe and 20 gauge (3.75 cm) needle and transferred (with needle removed) into a tube containing EDTA. The blood sample was maintained at ambient temperature (approximately 15–20 C) for approximately 10 days. The unclotted blood had sedimented. The plasma level was clear and the erthrocytes were at the bottom of the tube. Without agitating the tube, a small amount of plasma was withdrawn from the unstopped tube using a sterile Pasteur pipet. Plasma was examined for spirochetes under 400× using a light microscope. Spirochetes were seen within the plasma sample. The tube of blood was then sent to R. S. Lane (Department of Entomological Sciences, University of California, Berkeley, California, USA) for identification. Spirochetes were taken directly from the plasma and smeared onto a slide. The smear was stained using an Indirect Immunofluorescence Assay (IFA) using specific monoclonal antibody H5332 (Barbour et al., 1983) and FITC labeled goat anti-mouse serum. The spirochetes were identified as *B. burgdorferi*. All attempts to pass this isolate into BSK II media failed to establish a continuous culture line.

Whole brain, heart, spleen, and kidneys were fixed in 10% buffered formalin. Three histological sections from each organ were stained by Steiner's technique (Wright and Nielsen, 1990). The sections were examined under a light microscope at 400× and 1,000×. Only one spirochete was observed in each of the 12 histological sections. All spirochetes were morphologically similar to the spirochetes observed from the plasma sample.

Blood is a poor source for the isolation of *B. burgdorferi* (Wright and Nielsen, 1990; Kimsey and Spielman, 1990; Murray, 1990; Lane and Brown, 1991; Gordus, 1992). Spirochetemias with *B. burgdorferi* are very low in wild animals, whereas, with relapsing fever borreliae (*B. hermsii*), peak spirochetemias can reach 1×10^7 spirochetes/ml of blood (Barbour and Hayes, 1986).

Borrelia burgdorferi prefers the interstitial areas of tissue. Kimsey and Spielman (1990) suggested that *B. burgdorferi* prefers to disseminate via the intracellular matrix of ground substance of the epidermis. The spirochete's increased mobility appears to depend on the increased viscoelasticity (up to 1,200 centipoise) of the medium. The affinity of *B. burgdorferi*

to interstitial tissue probably explains why researchers have better success isolating *B. burgdorferi* from the kidneys and spleens (Anderson et al., 1986a, b, 1987a, b) and bladder tissue (Callister et al., 1988, 1989) of wild animals. The most successful technique for isolating *B. burgdorferi* from wild animals in California (Lane and Brown, 1991) is an ear biopsy (Sinsky and Piesman, 1989). Lane and Brown (1991) successfully isolated *B. burgdorferi* from 39% of 18 California kangaroo rats (*Dipodomys californicus*) and 37% of 82 dusky-footed wood rats using the ear biopsy technique, whereas no spirochetes were isolated from the blood or tissues.

Attempts to isolate *B. burgdorferi* from over 1,000 blood and tissue samples from wild animals in California have been unsuccessful (Lane and Brown, 1991; Gordus, 1992). Only once has *B. burgdorferi* been isolated from the blood of another wild animal in California. This animal was a dusky-footed wood rat (*N. fuscipes*) captured in southern California (Boyce et al., 1992). Attempts to isolate spirochetes from this same individual wood rat using an ear biopsy was unsuccessful.

Presently, the only known *Ixodes ricinus* complex tick that inhabits Tule Lake NWR is *I. angustus* (Gordus, 1992). *Ixodes pacificus* and *I. neotomae* are most commonly found in the coastal mountains and in the foothills of the Sierra Nevada Mountains (Furman and Loomis, 1984). Tule Lake NWR is outside the distribution range of these two ticks.

California kangaroo rats and dusky-footed wood rats have been suggested as sylvatic reservoirs for *B. burgdorferi* in California (Lane and Brown, 1991; Brown and Lane, 1992; Boyce et al., 1992). California kangaroo rats and dusky-footed wood rats have geographical ranges that partially overlap the geographical range of the bushy-tailed wood rat. The California kangaroo rat is found in the Upper and Lower Sonoran Life Zones. The dusky-footed wood rat is primarily found in the Upper Sonoran and Transitional Life Zones on the western slope of the Sierra Nevada and the Coast Range from the Mexico-USA border to Oregon. The bushy-tailed wood rat overlaps the distribution of the former in the Transitional Life Zones in northern California (Ingles, 1965). These overlapping ranges include Tule Lake NWR. The preferred ecological niche of these two species of wood rats differ. The dusky-footed wood rat prefers chaparral or forested areas, living in lodges on the ground or in trees. The bushy-tailed wood rat prefers rimrock or rock slides building its den in the rock crevices (Burt and Grossenheider, 1976; Whitaker, 1980). The isolation of *B. burgdorferi* from a bushy-tailed wood rat suggests that this species may be a potential sylvatic reservoir host within another ecological niche in California.

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