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SUSCEPTIBILITY OF SELECTED RODENT SPECIES FROM COLORADO TO *BORRELIA BURGDORFERI*

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ABSTRACT: To determine the susceptibility of some common Colorado (USA) rodent species to *Borrelia burgdorferi*, pregnant *Peromyscus maniculatus*, *Tamias minimus*, and *Spermophilus lateralis* were trapped in May 1990 and kept in quarantine until their young were old enough to be used in the experiment. Six to eight 8-wk-old individuals of each of the Colorado species and, for comparison, eight laboratory raised *P. leucopus* were subcutaneously inoculated with $\geq 10^5$ spirochetes in 0.1 ml in July 1990. Tissue specimens were collected for isolation from these animals through April 1991. Spirochetes were isolated from blood, ear, bladder, kidney, spleen, liver, and eye in Barbour-Stoener-Kelly (BSK) medium from *P. maniculatus*, *P. leucopus* and *T. minimus*. Spirochetes were isolated from at least one tissue from all of these animals and no isolations were obtained from any of the *S. lateralis*. Thus, three of the four rodent species tested are susceptible to, and could harbor, *B. burgdorferi*.

Key words: *Borrelia burgdorferi*, Colorado, *Peromyscus maniculatus*, *Peromyscus leucopus*, *Tamias minimus*, *Spermophilus lateralis*, susceptibility.

INTRODUCTION

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the leading vector-borne disease of humans in the United States and cases have been reported from 46 states, although it is considered endemic for humans in only 18 states in three regions (Dennis, 1991). Some human cases have been reported from the Rocky Mountain states, but the disease's endemic status there has not been established. However, an enzootic cycle of *B. burgdorferi* has recently been described in northern Colorado (USA) (Maupin et al., 1994).

One of the most interesting aspects of Lyme disease is the wide host range of *B. burgdorferi*. This spirochete has been isolated from and is known to cause disease not only in humans (Steere et al., 1983) but also in numerous animal species, including monkeys (Philipp et al., 1994), domestic pets (Burgess, 1986), and farm animals (Bosler et al., 1988). It has also been isolated from various wild mammals and birds (Anderson, 1988).

Small mammals such as white-footed mice (*Peromyscus leucopus*), deer mice (*P. maniculatus*), eastern chipmunks (*Tamias striatus*), and meadow voles (*Microtus pennsylvanicus*), are important reservoirs of *B. burgdorferi* and hosts for immature vector ticks, *Ixodes scapularies* (Anderson et al., 1983; Callister et al., 1991). The isolation of the Lyme disease spirochete from the tissues of mammals (Anderson et al., 1985) or ticks (Kocan et al., 1992) is crucial for identifying *B. burgdorferi* enzootic foci. *Borrelia burgdorferi* has been isolated from kidneys, spleen, liver, testes, blood, bladder, brain, fetus, and eyes (Anderson et al., 1983, 1985; Duray and Johnson, 1986; Ubico, 1992) of mammals and birds.

Evidence for the presence of *B. burgdorferi* in Colorado was established in 1992 (Ubico, 1992) and its presence was confirmed in northern Colorado in 1994 when specific vertebrate host and vector species were identified (Maupin et al., 1994). Our objective was to determine whether three common species of Colorado rodents were susceptible to *B. burgdorferi*.

MATERIALS AND METHODS

During May 1990, pregnant rodents native to Colorado were collected. Sherman (8 × 9 × 23 cm, H.B. Sherman Traps, Inc., Tallahassee, Florida, USA) and small-sized, wire live traps (13 × 13 × 41 cm, Tomahawk Live Trap Com-

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pany, Tomahawk, Wisconsin, USA), baited with cracked oats were randomly placed in old coniferous forests in Big Thompson Canyon (40°24′N, 105°24′W) and Cherokee Park (40°52′N, 105°30′W) in north-central Colorado. Seventy-five traps were set for three nights at each collection site. The traps were checked and rebaited every morning. Trapped animals were collected and transported to the laboratory of the Centers for Disease Control in Fort Collins, Colorado, where they were transferred to a laboratory cage and kept for quarantine in isolation. The initial bedding provided in each cage was treated with 0.5% permethrin (Permanone Tick Repellent, Coulston International Corp., Easton, Pennsylvania, USA) to destroy ectoparasites on the animals. The pregnant animals and their young were held for a longer quarantine period until the young were weaned and old enough (8-wk old) to be used in the experiment. Adult males and females were returned to their respective natural habitats.

Six to eight young animals of each of the following species were tested: least chipmunk (*Tamias minimus*), golden-mantled ground squirrel (*Spermophilus lateralis*), and deer mouse. In addition, a group of laboratory-reared white-footed mice (Peromyscus Genetic Stock Center, University of South Carolina, Columbia, South Carolina, USA) was tested for comparison to the other rodent species. Rodents were housed in plastic cages with wood shavings and raw cotton for bedding and were provided with water and standard laboratory mouse chow ad libitum. At the completion of the experiment, all the animals were euthanized with carbon dioxide gas, post-mortem examination was performed and tissues were collected for culturing of spirochetes.

The primary isolate (NY90-14) of *B. burgdorferi* used to inoculate animals was obtained from the ear tissue of *P. leucopus* collected in the hyperendemic area of Westchester, New York (USA) (McLean et al., 1993). The isolate and two passages were propagated in Barbour-Stoener-Kelly (BSK) medium (Barbour, 1984) incubated at 34 C, divided into aliquots, and cryopreserved as described by Anderson (1989). The isolate was identified as *B. burgdorferi* by an indirect fluorescent antibody test with species-specific anti-OspA monoclonal antibody H5332 (Barbour et al., 1986).

Animals were inoculated subcutaneously with a second-passage culture of live *B. burgdorferi* (NY90-14) suspended in 0.1 ml of BSK culture medium containing >100,000 spirochetes per ml. Dosage was estimated from counts of spirochetes in serially diluted volumes of live cultures.

Rodents were anesthetized with methoxyflurane (Metofane, Pittman-Moore, Inc., Mundelein, Illinois, USA) for handling. Pre-inoculation blood samples (0.2 ml) were obtained by capillary pipet from the suborbital sinus on all animals including the mothers of any young used. Daily blood samples (0.05 ml) were taken from all inoculated animals, starting 24 hr post-inoculation (PI), during the first week for determination of spirochetemia. Several drops of blood from the capillary tubes were shaken directly into tubes of BSK medium and incubated at 34 C.

Tissue samples were obtained weekly from the ears of infected animals for 7 wk following inoculation. The ear surface was thoroughly cleaned with 70% ethyl alcohol; and a small piece of tissue (3 to 4 mm) was collected with a punch (Sinsky and Piesman, 1989). The tissue specimen was first dipped in 5% hydrogen peroxide, then in 70% ethyl alcohol, and then rinsed with sterile phosphate-buffered saline (PBS) before being placed in tubes of BSK medium.

Isolation of live spirochetes from the rodents was accomplished at necropsy in the following manner. A portion of each organ was removed aseptically and placed into prelabeled, individual, sterile plastic bags, which were kept at 4 C and processed within 2 hr after collection. Each specimen was aseptically removed from the plastic bag, rinsed in 70% ethyl alcohol, then in 5% hydrogen peroxide, and finally in PBS. A 3 mm^3 piece of tissue was then placed into BSK medium. The remaining tissue was placed in a labeled, sterile screw-capped vial containing BSK and 30% glycerol and frozen at −70 C.

The cultures were kept at 33 to 34 C and observed by darkfield microscopy once a week for the presence of spirochetes. Negative cultures were examined for up to 8 wk. For positive cultures, in which spirochetes and spirochete-like organisms were observed by darkfield microscopy, slides were prepared and tested with a direct fluorescent antibody test (Lord et al., 1992) by using a fluorescein-labeled, goat anti-*Borrelia* sp. antibody conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA).

The chi-square (χ^2) and Fisher's exact tests were used to statistically analyze the laboratory data (Sokal and Rohlf, 1981).

RESULTS

All rodent species, except *S. lateralis*, were susceptible to inoculation with *B. burgdorferi* and developed detectable spirochetemias and tissue infections. Control

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TABLE 1. Isolation of spirochetes in Barbour-Stoenner-Kelly culture media from bloods of least chipmunks (*Tamias minimus*), deer mice (*Peromyscus maniculatus*), and white-footed mice (*P. leucopus*) inoculated with *Borrelia burgdorferi*.

Days post-inoculation	*T. minimus*		*P. maniculatus*		*P. leucopus*	
	Positive/tested	%	Positive/tested	%	Positive/tested	%
0	0/6[a]	0[b]	0/8	0	0/8	0
1	4/6	67	1/8	13	3/8	38
2	0/6	0	5/8	63	3/8	38
3	3/6	50	5/8	63	3/8	38
4	4/6	67	2/8	25	0/8	0
5	3/6	50	0/8	0	0/8	0
6	1/6	17	0/8	0	0/8	0
7	1/6	17	0/8	0	0/8	0
8	1/6	17	3/8	38	0/8	0
Total	6/6	100	8/8	100	7/8	88

[a] Number positive/number tested.
[b] Percent positive.

rodents, which either were inoculated with BSK culture medium or not inoculated, did not develop spirochetemia and no spirochetes were isolated from any of their tissues.

Spirochetemias were detected beginning 24 hr PI in four of six *T. minimus*, one of eight *P. maniculatus*, and three of eight *P. leucopus* (Table 1). All *T. minimus* and *P. maniculatus* had spirochetemias by day 4 PI. Only one *T. minimus* remained spirochetemic for the first 8 days. However, *B. burgdorferi* was isolated from blood samples taken from two different *T. minimus* at the time of necropsy (Table 3). Three *P. maniculatus* remained spirochetemic for the first 8 days PI (Table 1), but none were found with circulating *B. burgdorferi* in the blood at the time of necropsy (Table 3). Six of eight *P. leucopus* had developed spirochetemia by day 3 PI. *Borrelia burgdorferi* could not be isolated from blood samples of any living *P. leucopus* after day 4 PI (Table 1) although at

TABLE 2. Isolation of spirochetes in Barbour-Stoenner-Kelly culture media from ear tissue of least chipmunks (*Tamias minimus*), deer mice (*Peromyscus maniculatus*), and white-footed mice (*P. leucopus*) inoculated with *Borrelia burgdorferi*.

Days post-inoculation	*T. minimus*		*P. maniculatus*		*P. leucopus*	
	Positive/tested	%	Positive/tested	%	Positive/tested	%
0	0/6[a]	0[b]	0/8	0	0/8	0
7	0/6	0	0/8	0	0/8	0
14	2/6	33	4/8	50	4/8	50
21	6/6	100	6/8	75	8/8	100
28	6/6	100	7/8	88	7/8	88
35	6/6	100	7/8	88	6/8	75
42	6/6	100	4/8	50	3/8	38
49	6/6	100	0/8	0	0/8	0
72	6/6	100	NT[c]	—	NT	—
234	NT	—	5/8	63	NT	—
292	NT	—	NT	—	5/8	63
Total	6/6	100	7/8	88	8/8	100

[a] Number positive/number tested.
[b] Percent positive.
[c] NT = not tested.

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TABLE 3. Isolation of spirochetes in Barbour-Stoenner-Kelly culture media from tissues collected at time of necropsy (72, 234, and 290 days postinoculation respectively) of least chipmunks (*Tamias minimus*), deer mice (*Peromyscus maniculatus*), and white-footed mice (*P. leucopus*) inoculated with *Borrelia burgdorferi*.

	T. minimus		*P. maniculatus*		*P. leucopus*	
Tissue	Positive/ tested	%	Positive/ tested	%	Positive/ tested	%
Blood	2/6[a]	33[b]	0/8	0	2/8	25
Ear	6/6	100	5/8	63	5/8	63
Liver	0/6	0	1/8	13	0/8	0
Spleen	2/6	33	5/8	63	1/8	13
Kidney	1/6	17	1/8	13	0/8	0
Bladder	5/6	83	7/8	88	1/8	13
Eye	2/6	33	6/8	75	4/8	50
Total	6/6	100	8/8	100	8/8	100

[a] Number positive/number tested.
[b] Percent positive.

the time of necropsy spirochetes were isolated from blood samples taken from two mice (Table 3). Spirochetemias were unequal among the three species, but spirochetes were isolated from the blood on at least one occasion from all individuals inoculated, except from one *P. leucopus*.

Borrelia burgdorferi was isolated from ear tissue of two of six *T. minimus*, four of eight *P. maniculatus*, and four of eight *P. leucopus* by day 14 PI (Table 2). By day 21 PI, all of the ear tissue cultures from *T. minimus* were positive, and spirochetes were isolated from ear tissue at the time of necropsy (Tables 2, 3). *Borrelia burgdorferi* spirochetes also were isolated from ear tissue of seven of eight *P. maniculatus* by day 28 PI (Table 2) and all eight *P. leucopus* by day 21 PI (Table 2); five of eight individuals in both species had spirochetes in ear tissue at necropsy 234 and 292 days PI, respectively (Table 3). Spirochetes were isolated from ear tissue on at least one occasion from all rodents inoculated except from one *P. maniculatus* that died during the experiment.

At the time of necropsy, *B. burgdorferi* also was isolated from the liver, spleen, eye, kidney, and bladder of these infected rodents (Table 3). There was a significantly, higher isolation prevalence of spirochetes from both spleen ($P < 0.05$) and bladder ($P < 0.01$) tissues from *P. maniculatus* than from *P. leucopus* at the time of necropsy, but there were no other statistically significant differences.

One *P. maniculatus* was found dead 140 days PI. Very low numbers of *B. burgdorferi* had been isolated from its blood samples on day 1 and 2 PI, and no isolates were recovered from ear tissue samples (Table 2). However, very high counts of spirochetes were obtained from the cultures of kidney and bladder tissue taken at necropsy from this animal. Skin lesions did not develop at the site of injection on any of the rodents.

Ten inoculated rodents became hyperactive, but had no clinical signs of disease such as lameness, paralysis, inactivity, or reduction in food intake during the study. At necropsy, the animals were observed to be well-nourished with normal appearing fur coats and no swelling of limb joints. No abnormal fluid was found in the body cavities, including the pericardial sac. No gross abnormalities were seen in the thoracic and abdominal viscera.

DISCUSSION

According to Burgdorfer and Mavros (1970), *S. lateralis* is refractory to *B. hermsii* infections, and the results from this study provide evidence that this species also may be refractory to *B. burgdorferi* because no demonstrable spirochetemias

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or tissue infections developed. Based on these results, *P. maniculatus* and *T. minimus* are as susceptible to *B. burgdorferi* as is the well-established rodent host species, *P. leucopus,* and we believe that these rodent species could be important reservoirs for this disease agent in Colorado where *P. leucopus* is limited or absent.

The *B. burgdorferi* spirochetemias in wild rodents infected during this study occurred during the first few days PI as was described previously for other rodent species by Duray and Johnson (1986) and McLean et al. (1993). It was possible to isolate *B. burgdorferi* from blood taken 24 hr PI from chipmunks, deer mice, and white-footed mice. McLean et al. (1993) previously isolated spirochetes from blood taken 24 hr PI from eastern chipmunks which had been inoculated with the same strain of *B. burgdorferi.* Spirochetemias were detected mostly during the first 5 days PI. In our study, *B. burgdorferi* was not isolated from blood samples after the first 8 days PI; although Johnson et al. (1984) isolated spirochetes from the blood of hamsters (*Mesocricetus auratus*) up to but not more than 2 wk. Thus, following the initial infection, *B. burgdorferi* may spread via the blood to other body sites during the first week of infection and then disappear from the blood. This is further supported by the observation that these microorganisms usually were not isolated from ear tissue until 7 to 14 days PI. However, *B. burgdorferi* was recovered during necropsy from blood samples at 72 days PI from *T. minimus* and 290 days PI from *P. leucopus,* but not at 237 days PI from *P. maniculatus.* Duray and Johnson (1986) isolated *B. burgdorferi* from blood from the left ventricles of the heart of hamsters 6.5 and 7 mo PI. Thus we believe *B. burgdorferi* can persist chronically in the host and that the host retains the capacity for relapsing spirochetemias for a relatively long period.

Borrelia burgdorferi was not isolated from urine samples from any of the experimental rodents even though, according to Bosler and Schulze (1986), the prevalence of spirochetes in the urine of feral reservoir hosts regularly is higher than their prevalence in the blood. However, spirochetes consistently were isolated from the bladder of the experimental rodents in this study even after the animals had died. These results are in agreement with the previous findings of Schwan et al. (1988) and Callister et al. (1989). Based on these results, not only the bladder but also the kidneys harbor *B. burgdorferi.* Bosler and Schulze (1986) suggested that the kidney concentrates or filters these microorganisms from the blood and that they then enter the urine alive.

Infection of various internal organs was documented by isolation of *B. burgdorferi* in BSK culture media. The isolation of these microorganisms from the eyes of three species is evidence that rodents may suffer ocular manifestations of *B. burgdorferi* infection. This phenomenon has been reported in humans (Flach and Lavoie, 1990). Duray and Johnson (1986) previously had detected *B. burgdorferi* in the eyes of hamsters by culture and histologic examination and suggested that the microorganism's attraction to the eye is related to the viscosity of the vitreous chamber and the chamber's glucose and electrolyte contents.

From these results, we propose that the deer mouse and least chipmunk also may be potential reservoir host species for *B. burgdorferi* in Colorado. These two host species are in addition to the Mexican wood rat (*Neotoma mexicana*) and the rock mouse (*P. difficilis*), from which this spirochete was recently isolated in northern Colorado (Maupin et al., 1994). The presence, distribution, and description of enzootic transmission cycles of *B. burgdorferi* in other locations of the Rocky Mountain region need to be determined.

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