NATURAL AND EXPERIMENTAL BORRELIA BURGDORFERI INFECTIONS IN WOODRATS AND DEER MICE FROM CALIFORNIA

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ABSTRACT: Sequestration of spirochetes and concurrent histopathologic lesions were evaluated in tissues of Borrelia burgdorferi-infected dusky-footed woodrats (Neotoma fuscipes) and deer mice (Peromyscus maniculatus). Rodents were either wild-caught and naturally infected or were experimentally infected by tick bite, by intramuscular (i.m.) injection of cultured spirochetes, or by i.m. injection of tick suspensions. Samples of host tissues, including skin, blood, ear, brain, eye, heart, lung, liver, spleen, kidney, and urinary bladder, were removed from up to 21 woodrats and four deer mice and cultured in BSK II medium. Borreliae-positive cultures of ear punch biopsies were obtained from 10 of 11 woodrats and from all of four deer mice. Additionally, positive cultures were obtained from three of 36 skin biopsies of woodrats, and from one of 36 cultures of woodrat blood. In contrast, spirochetes were not observed in 505 cultures of internal organs or whole blood. Samples of tissues from seven naturally infected woodrats, four experimentally infected woodrats, and nine experimentally infected deer mice also were examined for histopathologic lesions. Nonsuppurative cellular infiltrates were recognized in samples from most tissue types from woodrats, but few lesions were observed in tissues from deer mice. Recognized lesions in woodrats that were consistent with infections of Lyme borreliosis in other species included synovitis, myositis, and myocarditis. Such lesions were more common in woodrats than in deer mice. Inflammatory lesions, especially synovitis, were more common in woodrats with long-term infections than in woodrats with relatively short-term infections. No clinical signs of disease were observed in either species of rodent.

Key words: Borrelia burgdorferi, isolation, histology, Lyme disease, reservoirs, rodents.

INTRODUCTION

The etiologic agent of Lyme disease, Borrelia burgdorferi, is maintained in enzootic transmission cycles involving wildlife reservoirs throughout much of the temperate world. Primary reservoir hosts of B. burgdorferi include the white-footed mouse (Peromyscus leucopus) in the northeastern and upper midwestern states (Mather et al., 1989), and the dusky-footed woodrat (Neotoma fuscipes) (hereinafter referred to as woodrats), in California (Brown and Lane, 1992). The geographic range of the deer mouse (Peromyscus maniculatus) extends throughout most of the United States and this species is undoubtedly exposed to B. burgdorferi in some habitats of the northeast, upper midwest and far west (Callister et al., 1988; Lane, 1990). However, the deer mouse has not been implicated as a primary reservoir host in any region.

One criterion for determining the relative reservoir potential of host species for B. burgdorferi depends upon the prevalence of infection in populations of different species of hosts. Lyme disease spirochetes have been associated previously with many species of potential reservoir hosts, and most often isolated from the urinary bladders, kidneys, spleens, and ear-punch biopsies of wild rodents (Anderson et al., 1985; Schwan et al., 1988; Callister et al., 1989; Lane and Brown, 1991). The comparison of isolation attempts from different species of hosts, or among hosts of different geographic regions, relies on the assumption that the probability of culturing spirochetes from host-tissues is independent of host species and of locally predominate strains of spirochetes. The validity of such assumptions currently is unknown.

Lyme borreliosis may affect multiple organ systems with pathologic changes of varying severity (Duray and Steere, 1986; Preac Mursic et al., 1990; Barthold et al.,...
1991). The skin, skeletal system, nervous system, and heart typically are associated with chronic disease, and infiltration of other organs by inflammatory cells (primarily lymphocytes, macrophages, and plasma cells) has been reported often. Histopathologic lesions caused by infections of *B. burgdorferi* have been reported previously in laboratory rats and mice (Schaible et al., 1990; Barthold et al., 1991), hamsters (Duray and Johnson, 1986; Hejka et al., 1989), and gerbils (Preac-Mursic et al., 1990). Nonsuppurative cellular infiltrates in the brains, kidneys, livers, and lungs of wild-caught *P. leucopus* were associated with *B. burgdorferi* in one study (Burgess et al., 1990), but not in others (Burgess et al., 1986; Wright and Nielsen, 1990).

Knowledge of infection and disease in reservoir hosts adds to the understanding of natural systems of maintenance and transmission. Although studies of *B. burgdorferi* infections in *P. leucopus* have been reported, such evaluations are lacking for reservoirs from other regions. Our objectives were to compare the relative sequestration of *B. burgdorferi* in tissues of woodrats and deer mice and to evaluate tissues from both rodents for histologic evidence of *B. burgdorferi*-related lesions.

**MATERIALS AND METHODS**

We used six groups of experimental rodents (*n* = 32; including two groups of wild-caught woodrats with naturally-acquired *B. burgdorferi* infections (groups 1 and 2), woodrats with naturally acquired long-term infections of >1 yr duration (group 3), woodrats with short-term experimental infections (group 4), and deer mice with long (group 5) and intermediate (group 6) durations of infection. All rodents used in these studies were live-trapped, or were from colonies whose founders were live-trapped, at the University of California Hopland Field Station (39°00'N, 123°00'W) in Mendocino County, California (USA) in May and June 1989 (group 1) or March to June 1990 (all other groups).

Wild adult woodrats of unknown history (group 1, *n* = 10; group 2, *n* = 2) were trapped in spring and winter with wire-mesh live traps (Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA, and National Brand Live Trap Company, Tomahawk, Wisconsin), and returned to animal facilities at the University of California, Berkeley, California. Woodrats were necropsied aseptically, and their tissues were cultured in modified Barbour-Stoenner-Kelley medium (BSK II) (Barbour, 1984) containing 50 μg/ml rifampicin (Sigma Chemical Company, St. Louis, Missouri, USA) (hereinafter referred to as medium).

Pregnant woodrats were trapped in spring (late March through the middle of June) and maintained separately in captivity where they gave birth to and reared their offspring. Many of these females were infected naturally with *B. burgdorferi* prior to capture and maintained their infections throughout the period of their captivity as determined by cultures of ear-punch biopsies taken on the day of capture and periodically thereafter. Five of these naturally infected female woodrats (group 3) were necropsied 13 to 15 mo after capture. Group 4 consisted of four yearling woodrats that were born and raised in captivity by the group 3 females. Group 4 woodrats were necropsied approximately 80 days following experimental infection with *B. burgdorferi* by infected tick bite. The offspring discussed herein remained uninfected, as determined serologically and by periodic culture of ear-punch biopsies, until approximately 7-mo old when they were experimentally exposed to *B. burgdorferi*-infected *Ixodes neotoma* nymphs (Brown and Lane, 1992).

Six deer mice (group 5) were selected from a spirochete-free colony. These mice were injected intramuscularly (i.m.) with 10³ spirochetes (*B. burgdorferi* isolate CA 11, passage level 2) in approximately 0.035 ml medium. Isolate CA 11 was originally isolated from an *Ixodes pacificus* tick collected in Mendocino County, California (Lane and Pascocello, 1989). Four of the six mice survived 13 mo following experimental infection.

Five additional clean-colony deer mice (group 6) each were inoculated i.m. with ≤0.2 ml of a 1-ml suspension of the tissues of a replete *I. pacificus* female. Borreliae cultured from this tick were identified later as *B. burgdorferi* (identification of borreliae described below), and the isolate was designated number CA 172. Group 6 mice were observed for signs of disease, including inappetence, lethargy, lameness, and signs of neurologic disorder, for 40 days following inoculation. Group 6 deer mice were necropsied 153 days after injection, and their tissues were saved for histologic examination; ear-punch biopsies of these mice were cultured on two dates prior to necropsy. Borreliae were cultured from ear-punch biopsies of group 6 deer mice following inoculation, but no spirochetes were cultured from the biopsies taken during necropsies of these mice.

Tissues from two woodrats served as controls
in histopathologic comparisons. These woodrats were young adults born in captivity and maintained like the other woodrats, but they were not exposed to ticks or *B. burgdorferi*. Likewise, three clean-colony deer mice were necropsied and their tissues were compared histologically to those of the nine deer mice in groups 5 and 6.

Rodsents were euthanized by inhalation overdose with methoxyflurane (Pitman-Moore, Inc., Mundelein, Illinois, USA). The fur and skin of each animal was scrubbed prior to necropsy with Betadine (The Purdue Frederick Company, Norwalk, Connecticut, USA) and 70% ethanol, and aseptic techniques were used throughout the necropsy procedures. One drop of whole blood, drawn retro-orbitally or by cardiac puncture, was inoculated into a microcentrifuge tube containing 1.25 ml medium. Additionally, whole blood from 11 woodrats (groups 2, 3, and 4) was centrifuged, serum removed, and single drops of the cells at the surface of remaining pellets were added to 1.25 ml medium. Two 2-mm punch biopsies, one from the base of each ear of rodents from groups 2 through 5, and eight 2-mm skin-punch biopsies, two each from the shaved throats, backs, axillae, and tails of woodrats in groups 3 and 4, were inoculated directly into 1.25 ml of medium. Internal organs were evaluated somewhat differently in that three samples were cultured rather than one. The biopsies of internal organs, which ranged in size from one-half of the spleens of mice to approximately 4 mm\(^3\) for internal organs of woodrats, including right eyes, brains (equal portions of cerebrum and cerebellum), hearts, lungs, livers, spleens, kidneys, and bladders, were triturated in 2 ml medium using porcelain mortars and pestles. Three ml of medium was added to solutions containing triturated organs and the resulting slurries were allowed to clarify for 30 min. One large drop of each slurry then was inoculated into separate vials containing 1.25 ml medium. Additionally, 0.5 ml of each slurry was inoculated into 5 ml medium, mixed thoroughly, and a 1:10 dilution of this mixture was prepared. This design resulted in one 1.25-ml culture each of whole blood, pelleted blood cells, ear-punch biopsies, and skin biopsies, and three cultures each (one 1.25-ml and two 5-ml) of all other organs.

Cultures were incubated at 34 C and examined weekly by dark-field microscopy for \(\geq 5\) wk. Positive cultures were confirmed to be *B. burgdorferi* by their reactivities with *B. burgdorferi*-specific monoclonal antibodies (MAbs; H5332, H5TS, H6831, H3TS) using an indirect immunofluorescence assay, and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Lane and Pascollo, 1989).

Tissues from each rodent, including brain (fore-, mid-, and hindbrain), heart, lung, liver, spleen, kidney, urinary bladder, left eye, ear pinna, and the right front and rear legs, were immersion-fixed in phosphate-buffered forma- lin and submitted to California Veterinary Diagnostics, Inc. (Sacramento, California). Joints were demineralized, and tissue samples were embedded in paraffin, sectioned at 6 \(\mu m\), and stained with hemotoxylin and eosin.

Fisher’s exact test (Sokal and Rohlf, 1981) was used to compare the results of culturing ear-punch biopsies, skin biopsies, and pelleted blood as well as the prevalence of synovitis in woodrats with long-term versus relatively short-term infections.

RESULTS

Tissues from 21 infected woodrats (groups 1 to 4) and four deer mice (group 5) were cultured for spirochetes. Borreliae were detected more often in ear-punch biopsy cultures (10 of 11) than in cultures of skin biopsies (three of 36) or cultures of pelleted blood cells (one of 11) from samples taken from woodrats at necropsy (Fisher’s exact test; \(P = 0.0000\) and \(P = 0.0002\), respectively) (Table 1). Likewise, spirochetes grew in all of four ear-punch biopsies taken from the group 5 mice. Spirochetes were not observed in any cultures (\(n = 505\)) of whole blood or of the internal organs from any of the rodents. All spirochetes cultured from naturally-infected woodrats were identified as *B. burgdorferi*.

Minimal to moderate non-suppurative infiltrates were observed in all organs examined from *B. burgdorferi*-infected woodrats except brain (Table 2). Lesions observed in woodrat tissues included multifocal, minimal to mild dermatitis of the ears characterized by lymphocytes, plasma cells and occasional eosinophils; multifocal, minimal to mild synovitis in knee joints, with inflammation of the striated muscle and skin adjacent to affected joints in one animal; focal, minimal to mild conjunctivitis; multifocal myocarditis; minimal interstitial pneumonia with or without mild peribronchitis (observed in one woodrat in group 3 only); multifocal cholangitis; hemosiderosis; minimal to mild
### Table 1. Results of culturing tissues from woodrats (*Neotoma fuscipes*) and deer mice (*Peromyscus maniculatus*) infected with *Borrelia burgdorferi*.

<table>
<thead>
<tr>
<th>Group (species)</th>
<th>Number sampled</th>
<th>Status, route of infection</th>
<th>Duration of infection</th>
<th>Ear punch biopsy</th>
<th>Skin</th>
<th>Whole blood</th>
<th>Blood pellet</th>
<th>Internal organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N. fuscipes)</td>
<td>10</td>
<td>Wild caught; naturally infected</td>
<td>Unknown</td>
<td></td>
<td></td>
<td>0/10⁺</td>
<td>0/40</td>
<td>0/40</td>
</tr>
<tr>
<td>Group 2 (N. fuscipes)</td>
<td>2</td>
<td>Wild caught; naturally infected</td>
<td>Unknown</td>
<td>2/2</td>
<td></td>
<td>0/2</td>
<td>1/2</td>
<td>0/16</td>
</tr>
<tr>
<td>Group 3 (N. fuscipes)</td>
<td>5</td>
<td>Wild caught; naturally infected</td>
<td>≥13–15 mo</td>
<td>4/5, 0/5, 0/5, 0/5, 1/5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/40</td>
</tr>
<tr>
<td>Group 4 (N. fuscipes)</td>
<td>4</td>
<td>Laboratory colony; tick bite</td>
<td>≈80 days</td>
<td>4/4, 0/4, 1/4, 1/4, 0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/32</td>
</tr>
<tr>
<td>Group 5 (P. maniculatus)</td>
<td>4</td>
<td>Laboratory colony; intramuscularly injected</td>
<td>13 mo</td>
<td>4/4</td>
<td></td>
<td>0/4</td>
<td>0/4</td>
<td>0/32</td>
</tr>
<tr>
<td>All groups</td>
<td>25</td>
<td></td>
<td></td>
<td>14/15, 0/9, 1/9, 1/9</td>
<td>1/9</td>
<td>0/25</td>
<td>1/11</td>
<td>0/160</td>
</tr>
</tbody>
</table>

* Each fraction represented the combined results from three cultures of each of the internal organs from each rodent, including brain, eye, heart, lung, liver, spleen, kidney, and urinary bladder except from group 1 in which only the last four tissues, respectively, were cultured.
*⁺ Number of positive cultures/number of biopsies from individual animals cultured.
* Injected intramuscularly with cultured *B. burgdorferi* isolate CA 11. Blank spaces occur where a procedure was not performed.
TABLE 2. Observed infiltrative lesions in organs removed from woodrats (Neotoma fuscipes) and deer mice (Peromyscus maniculatus) infected with *Borrelia burgdorferi*.

<table>
<thead>
<tr>
<th>Group (species)</th>
<th>Status, route of infection</th>
<th>Number of organs with lesions/number of animals examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 (N. fuscipes)</td>
<td>Wild caught; naturally infected</td>
<td>Skin: NE 1/2 0/2 0/2 1/2 1/2 2/2 0/2 2/2 0/2</td>
</tr>
<tr>
<td>Group 3 (N. fuscipes)</td>
<td>Wild caught; naturally infected</td>
<td>Joint: 4/5 5/5 0/5 1/5 1/5 2/5 4/5 0/5 5/5 0/4</td>
</tr>
<tr>
<td>Group 4 (N. fuscipes)</td>
<td>Laboratory colony; tick bite</td>
<td>Brain: 2/4 1/4 0/4 0/4 2/4 0/4 3/4 1/4 4/4 2/4</td>
</tr>
<tr>
<td>Group 5 (P. maniculatus)</td>
<td>Laboratory colony; intramuscularly injected</td>
<td>Eye: NE 0/4 0/4 1/4 0/4 1/4 0/4 0/4 0/4 0/4</td>
</tr>
<tr>
<td>Group 6 (P. maniculatus)</td>
<td>Laboratory colony; intramuscularly injected</td>
<td>Lung: NE NE NE 0/5 0/5 0/5 0/5 1/5 0/5 0/5</td>
</tr>
<tr>
<td>Controls (N. fuscipes)</td>
<td>Laboratory colony; uninfected</td>
<td>Liver: 0/2 0/2 0/2 0/2 0/2 1/2 0/2 2/2 0/2</td>
</tr>
<tr>
<td>Controls (P. maniculatus)</td>
<td>Laboratory colony; uninfected</td>
<td>Spleen: NE NE 0/3 0/3 0/3 0/3 0/3 1/3 0/3 0/3</td>
</tr>
<tr>
<td>Kidney</td>
<td>NE 0/3 0/3 0/3 0/3 0/3 0/3 0/3 0/3 0/3</td>
<td></td>
</tr>
</tbody>
</table>

* NE: Not examined.
1. Injected intramuscula1y with cultured CA 11.
2. Injected intramuscula1y with a tick suspension made from an infected *I. pacificus* female.

splenic lymphoid hyperplasia; and kidney disease (including minimal to mild interstitial nephritis, tubulointerstitial nephritis, mineralization, tubular dilatation, and interstitial collapse). Lesions were observed in tissues of the nine deer mice far less frequently than in those from woodrats; only four lesions, one case each of conjunctivitis, pneumonitis, cholangitis, and splenic lymphoid hyperplasia, were recognized in mice. Clinical signs of disease were not observed in any of the rodents.

**DISCUSSION**

Borreliae were isolated from ear-punch biopsies at necropsy from 14 of 15 rodents as well as from tail skin from the single woodrat whose ear-punch biopsy culture was negative. Surprisingly, spirochetes were not cultured from the internal organs of these rodents. These results are in sharp contrast to several studies in which urinary bladder, kidney, or spleen were found to be highly reliable sources of *B. burgdorferi*-infections in *P. leucopus* (Anderson et al., 1985, 1987; Schwan et al., 1988; Czub et al., 1992). Differential sequestration of spirochetes in host tissues may affect the disease process, as well as the infectivity of spirochetes for vector ticks.

Differences between our results and those from previous reports represent an unidentified difference in the host-parasite interaction. Several factors may have influenced our results. First, the ability to isolate spirochetes from different tissues may be related to the duration of infection (Barthold et al., 1991). However, we isolated *B. burgdorferi* from ear-punch biopsies irrespective of the duration of infection. Second, the route of infection affects the course of infection (Barthold, 1991a) as well as the ability of ticks to acquire spirochetes while feeding (Piesman, 1993). The mice reported herein were inoculated with spirochetes and the woodrats were infected, either naturally or experimentally, by tick bite. Although the route of infection may have been partially responsible for the lack of observed histopathology in the mice, we observed comparable ear-punch biopsy results in
samples from both species. Third, the apparent differences in ability to isolate from different tissues and organs may represent real differences in sequestration of spirochetes resulting from characteristics of the borreliac strains or the hosts involved; strains of California *B. burgdorferi* vary antigenically (Brown and Lane, 1992), genetically (LeFebvre et al., 1990; Schwan et al., 1993), in their infectivity for xenodiagnostic ticks fed on infected hosts (Lane et al., 1994), and in their ability to be recultured from ticks or hosts (Brown and Lane, 1992; Schwan et al., 1993).

Although strains of *B. burgdorferi* from California were more variable antigenically than strains from the northeastern United States (Brown and Lane, 1992), specific protein or genetic characteristics that separate California strains from those from other regions have not been reported (Bissett et al., 1987; Lane and Pascocello, 1989; Schwan et al., 1993). When isolates of *B. burgdorferi* from *Ixodes* spp. ticks from different geographic areas were compared, those from Europe and California were recultured less often from organs of experimentally infected rodents than were isolates obtained originally from the northeast and midwest (Barthold et al., 1990). Barthold et al. (1990) inoculated laboratory rats with one of 16 strains of *B. burgdorferi*, including four from California, and reported that spirochetes failed to grow in all of 78 cultures of tissues from rats inoculated with two of the four California isolates, and that borreliac grew in only two of 38 cultures from rats inoculated with a third isolate. However, the fourth isolate from California commonly was recultured from internal organs of rats (Barthold et al., 1990). In other studies, California tick-derived isolates of *B. burgdorferi* were recultured variably from the urinary bladders and spleens of inoculated mice (Schwan et al., 1988, 1993), and Schwan et al. (1993) suggested that strains of *B. burgdorferi* from California may be less infectious than isolates from other regions. Likewise, *B. burgdorferi* isolates cultured from ticks collected in California varied significantly in infectivity for hamsters, deer mice, and New Zealand white rabbits (Lane et al., 1994).

Lastly, interlaboratory variation in culture techniques may account for discrepant results among laboratories; different laboratories use slightly different techniques to culture borreliac. Our technique was most similar to that of Johnson et al. (1984), who suggested triturating tissues to a 10% (w/v) slurry and inoculating medium with supernatant following settling. Our inoculum consisted of both supernatant and small pieces of tissues. Schwan et al. (1988, 1993), Dorward et al. (1991), and Czub et al. (1992) triturated tissues in 1 ml of medium and inoculated the slurry directly into BSK II with or without antibiotics. The techniques of Anderson et al. (1985, 1987) and Callister et al. (1988, 1989) generally were similar, but differed in the variable use of 0.15% agarose, reducing agents, and additional antibiotics. These minor differences in techniques likely were not responsible for our inability to culture *B. burgdorferi* from visceral organs of deer mice or woodrats. Although urinary bladders proved efficacious for culturing *B. burgdorferi* from white-footed mice (Schwan et al., 1988; Callister et al., 1989; Czub et al., 1992) and culture of ear-punch biopsies proved effective for woodrats and deer mice (Lane and Brown, 1991; this report), we suggest that decisions concerning the culture of spirochetes from wild species of rodents be based on previous experiences involving the specific hosts and strains being studied.

Lesions observed in tissues from infected woodrats that were consistent with infiltrative lesions reported from other mammals infected with *B. burgdorferi* included synovitis, myocarditis, myositis, and possibly interstitial nephritis. Other lesions, such as tubular dilatation, nephrocalcinosis and interstitial collapse in kidneys, as well as mineralization of bladder tissue, were not associated previously with *B. burgdorferi* infections. Lesions recognized in woodrats were typically of minimal severity and were
characterized by focal or multifocal infiltrations of lymphocytes, plasma cells and macrophages. Although less severe, myocardial lesions of woodrats were consistent with lesions in other rodents having chronic heart infections of *B. burgdorferi* (Preac-Mursic et al., 1990; Armstrong et al., 1992). Immunocompromised laboratory animals have been shown to develop severe lesions of the heart, including mononuclear cell infiltration, deposition of collagen (Zimmer et al., 1990), and some involvement of multinucleated giant cell-syncytia (Schaible et al., 1990).

Synovitis has been associated frequently with Lyme borreliosis in both human and other animals. Severe synovitis and arthritis have been produced by inoculation of immunocompromised, infant, and inbred laboratory mice and rats (Schaible et al., 1989; Barthold et al., 1991, 1992) and by inoculation of the hind paws of hamsters (Schmitz et al., 1988; Hejka et al., 1989). However, only minimal to mild lesions were reported when synovitis was recognized in adult gerbils and in rodents from California (Preac-Mursic et al., 1990; this report).

Lameness in *B. burgdorferi*-infected *P. leucopus* has been reported, but the mice were not examined for synovitis or myositis (Burgess et al., 1987, 1990). Severe lameness might affect an individual rodent’s risk of predation, its ability to compete for mates, or the acquisition of sufficient food. No clinical signs of disease were observed in any of the woodrats or deer mice studied herein, but minimal to mild synovitis was relatively common in woodrats. Although the etiology of the observed lesions was not determined, the minimal synovitis observed in woodrats is the first report of synovitis from a naturally infected reservoir host infected with *B. burgdorferi*.

Myositis has been observed in muscles adjacent to inflamed joints both in humans (Atlas et al., 1988; Duray, 1989) and in rodents (Preac-Mursic et al., 1990; Munteanu et al., 1991). Inflammatory lesions were noted in striated muscle and skin adjacent to joints with synovitis in one of the woodrats.

Kidney lesions, including interstitial nephritis and tubulointerstitial nephritis, have been associated with borreliosis in a cow (Burgess et al., 1987), a dog (Grauer et al., 1988), and rodents (Preac-Mursic et al., 1990; Schaible et al., 1990). Interstitial and tubulointerstitial nephritis were recognized in *B. burgdorferi*-infected woodrats. However, a minimal interstitial nephritis was recognized in one of the control woodrats illustrating the fact that these lesions have multiple etiologies.

Borreliae were cultured from ear tissues of all infected animals, including the group 3 woodrat whose final ear punch biopsy culture was negative (Table 1) but whose ear-punch biopsy cultures taken prior to necropsy were positive (data not shown). However, the observed dermatitis of ear skin may have resulted from ticks having fed on the animals prior to necropsy rather than from the spirochetes themselves. Likewise, pneumonitis, although correlated with infections of *B. burgdorferi* previously (Barthold, 1991b), is a common problem among laboratory rodents and may have resulted from infection by other agents.

The number of lesions consistent with Lyme borreliosis was somewhat greater in the groups of adult woodrats with unknown duration of infection (group 2) and in those of long-term duration (group 3) than in those of short-term duration (group 4). This difference was attributable mainly to the recognition of synovitis in six of the seven woodrats of groups 2 and 3, compared to only one in four woodrats from group 4. Although this difference was not significant (Fisher’s exact test, *P* = 0.088), we propose that synovitis may be associated more often with long-term infections in woodrats.

Few lesions were recognized in the nine infected deer mice. Deer mice are susceptible to infection and maintain the agent for prolonged periods of time, but they appear relatively resistant to disease caused by *B. burgdorferi*. The ability to maintain
an agent for prolonged periods of time without suffering disease often is associated with primary reservoir species. Deer mice also are infested by vectors of *B. burgdorferi* and spirochete-infected individuals have been identified (Callister et al., 1988; Lane, 1990). However, deer mice have not been identified as an important reservoir host of *B. burgdorferi* in the northeastern, upper midwestern, or far western regions of the United States. The dusky-footed woodrat is an important reservoir species in California (Lane and Brown, 1991; Brown and Lane, 1992). Minimal to moderate inflammatory lesions were associated with infections of *B. burgdorferi* in woodrats, but what effect, if any, *Borrelia* has on individual fitness or woodrat populations remains to be determined.

Some of the observed lesions in *B. burgdorferi*-infected woodrats and deer mice appeared similar to lesions reported from other infected animals, and it seems likely that many of the lesions observed in woodrats were caused by *B. burgdorferi*. However, individual lesions must be interpreted with caution since no single lesion is pathognomonic for Lyme *Borrelia* infection. Furthermore, it is likely that some of the lesions observed in woodrat tissues, especially those of the livers and kidneys, had unrelated etiologies.

Samples from rodents were not assayed for exposure to other etiologic agents, and the field-caught, naturally-infected, woodrats were doubtless exposed to other pathogens in nature. Dusky-footed woodrats have been reportedly exposed naturally to at least nine viruses, a fungus, six bacteria, 13 protozoa, and seven helminths; significant references include, or are cited in Linsdale and Tevis (1951), Ryckman et al. (1981), and Milby and Reeves (1990). The extent to which some of these organisms interact, either directly or indirectly, influence the sequestration of *Borrelia* in different tissues, or cause histopathologic lesions similar to those caused by *B. burgdorferi* is unknown.

In summary, *B. burgdorferi* was cultured from ear-punch biopsies from 14 of 15 infected woodrats and deer mice and from skin biopsies or pelleted blood from woodrats. No spirochete-positive cultures were obtained from samples of eight internal organs from either species of host. We attribute these findings, which are in contrast to previous reports, to unidentified differences in the strains of *B. burgdorferi* or to differences in host-parasite interaction. Woodrats and deer mice were examined for histopathologic changes consistent with infections of *B. burgdorferi*. Lesions were seen more frequently in woodrats than in deer mice. Histopathologic lesions observed in woodrats that may have resulted from *B. burgdorferi* infections included myocarditis, myositis, synovitis, and potentially interstitial nephritis.

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**LITERATURE CITED**


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