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Author(s): Gary O. Maupin, Kenneth L. Gage, Joseph Piesman, John Montenieri, Steven L. Sviat, Lorna VanderZanden, Christine M. Happ, Marc Dolan and Barbara J. B. Johnson

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Discovery of an Enzootic Cycle of *Borrelia burgdorferi* in *Neotoma mexicana* and *Ixodes spinipalpis* from Northern Colorado, an Area Where Lyme Disease Is Nonendemic

Gary O. Maupin, Kenneth L. Gage, Joseph Piesman, John Montenieri, Steven L. Sviat, Lorna VanderZanden, Christine M. Happ, Marc Dolan, and Barbara J. B. Johnson

Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado

An intensive enzootic cycle of *Borrelia burgdorferi* was seen in populations of the Mexican wood rat, *Neotoma mexicana,* and *Ixodes spinipalpis* ticks in northern Colorado. Cultures of rodent ear tissue and ticks yielded 63 spirochetal isolates: 38 *N. mexicana,* 2 *Peromyscus difficilis,* and 23 *I. spinipalpis.* All 63 isolates were identified as *B. burgdorferi* sensu lato by polymerase chain reaction; a representative subset was characterized as *B. burgdorferi* by SDS-PAGE and immunoblotting. A tick-derived spirochete isolate was infectious to laboratory mice and *I. scapularis,* the principal vector of Lyme disease in endemic areas of the United States. The risk of human contact with infected *I. spinipalpis* appears to be minimal from this epidemiologically silent focus in northern Colorado, since this tick is restricted to wood rat nests in this semiarid environment.

Lyme disease is the leading vectorborne disease in the United States. Most human Lyme disease cases (≥80%) are reported annually from the Northeast and upper Midwest [1, 2]. The white-footed mouse, *Peromyscus leucopus,* is the primary reservoir of the Lyme disease spirochete, *Borrelia burgdorferi,* and is a major host for immature *Ixodes scapularis* ticks, the principal vector in these two areas [3–7]. In most other parts of the United States, the enzootic cycle of Lyme disease has not been fully elucidated. Nonetheless, recent reports have described reservoir competence of rodent species other than *P. leucopus* within the range of *I. scapularis.*

On Monhegan Island, Maine, the Norway rat, *Rattus norvegicus,* is the principal reservoir for *B. burgdorferi* and host for immature *I. scapularis* [8]. On Sapelo Island, Georgia, *B. burgdorferi* has been isolated from the cotton mouse, *Peromyscus gossypinus* [9]. On Isle au Haut, Maine, *Peromyscus maniculatus* is the major reservoir for *B. burgdorferi* and the principal host for immature *I. scapularis* [10]. In northwestern Illinois, the eastern chipmunk, *Tamias striatus,* has been shown to have a higher reservoir potential for *B. burgdorferi* than does the white-footed mouse in upland forest habitat [11].

In areas outside the distribution of *I. scapularis,* human Lyme disease is less frequently reported. In fact, Lyme disease is rare west of the Central Plains, except on the Pacific Coast [1]. In California and Oregon, the western blacklegged tick, *Ixodes pacificus,* has been identified as the primary vector of *B. burgdorferi* to wildlife and humans [12]. Studies in California have implicated the dusky-footed wood rat, *Neotoma fuscipes,* as an enzootic reservoir of *B. burgdorferi* [13]. This report of wood rat involvement in California prompted us to investigate the possibility of the presence of an enzootic Lyme disease cycle in northern Colorado, even though clinical evidence of human infections had not been recorded from this locale.

Materials and Methods

Study sites. Three undeveloped, nonresidential sites were surveyed for wood rats and ticks in the foothills of Larimer County, Colorado. The first site was on the western edge of Fort Collins at the Colorado State University Foothills Campus at an elevation of 1565 m. This terrain consists of slopes with rocky outcrops and scattered boulders of granite. The dominant vegetation is mountain mahogany, *Cercocarpus montanus,* and skunk brush, *Rhus trilobata.* The second site was 28.8 km south of Fort Collins on the east side of Carter Lake and was similar in elevation (1560 m), vegetation, and physiography to the Fort Collins site. The third site was 25.6 km north of Fort Collins at Owl Canyon and differed significantly from the other two sites. Owl Canyon is at an elevation of 1830 m with rocky outcrops and boulders of limestone and sandstone; the predominant vegetation is relict pinyon pine, *Pinus edulis,* also with some *C. montanus* and *R. trilobata.*

Collection of rodents and ticks. Rodents were trapped at the Fort Collins site in April, July, and September 1993 and at the other two sites in September 1993. One or two Tomahawk live traps (40.6 × 12.7 × 12.7 cm; Tomahawk Trap, Tomahawk,

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Reprints or correspondence: Dr. Gary O. Maupin, Division of Vector-Borne Infectious Diseases, NCID, CDC, P.O. Box 2087, Fort Collins, CO 80522.

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WI) baited with rolled oats were placed at active wood rat den sites in rocky habitats. Traps were set in the afternoon and checked the following morning for 2 consecutive days. On one occasion in July at the Fort Collins site, Sherman traps (23.3 × 8.8 × 7.5 cm; Sherman Trap, Tallahassee, FL) were used to supplement the larger Tomahawk traps. Captured rodents were anesthetized with methoxyflurane and examined for ticks, and ear tissue biopsy samples were taken. Ticks were removed from hosts, identified by using standard taxonomic keys [14–16], and compared with preserved museum specimens in our reference collection. Animals were released at the point of capture on the same day they were collected.

During May at the Fort Collins site, surveys were conducted for host-seeking ticks inside and within 10 m outside four active wood rat dens. Vegetation, rock, and soil substrates were sampled for ticks within the 10-m radius of nests by using a 1-m^2 cloth drag [17]. Distances of up to 2 m inside nest entrances were sampled for ticks by inserting a 90-cm^2 piece of flannel attached by an alligator clamp to a 2.2-m length of flexible wire cable. This device has been used extensively to collect host-seeking fleas from rodent burrows [18].

Isolation of spirochetes. We first attempted to isolate spirochetes from pooled and individual nymphal and adult ticks removed from wood rats in April at the Fort Collins site. Ticks were surface-disinfected in equal volumes of 0.5% iodine solution and 10% sodium hypochlorite for 30–60 min, rinsed with sterile PBS, soaked in 70% ethyl alcohol for 30 min, and rinsed again in sterile PBS. Ticks were then transferred to 4-mL glass tissue grinders and triturated in 0.5 mL of BSK-H culture medium (Sigma, St. Louis) [19] supplemented with 6% rabbit serum and antibiotics (rifampin, 50 μg/mL; phosphomycin, 200 μg/mL; cycloheximide, 200 μg/mL; and amphotericin B, 2.5 μg/mL). The resultant suspension was decanted into tubes containing 4 mL of BSK-H and incubated at 34°C, and cultures were examined for spirochetes by darkfield microscopy weekly for 4 weeks.

We evaluated the sensitivity of culturing spirochetes directly from ticks that were darkfield microscopy–positive for motile spirochetes. Nymphs and adults were surface-sterilized as above and placed in 2 drops of PBS on a microscope slide, and the midgut was dissected with heat-sterilized forceps. The excised gut contents were examined by darkfield microscopy for motile spirochetes. Coverslips were removed from positive slides, and 50 μL of PBS was added to the remaining suspension. Next, 25 μL was pipetted directly into BSK-H culture tubes. This procedure was done on 14 of 41 ticks examined by darkfield microscopy. After the coverslips were removed, all slides were air-dried, fixed with acetone, and stored at −20°C until they were examined by direct fluorescent antibody (DFA) microscopy.

Slides were treated with a 1:100 dilution of fluorescein isothiocyanate–conjugated antibodies; polyclonal antibody was produced in rabbits immunized with the Guilford strain of *B. burgdorferi* [17]. Ear tissue biopsy specimens were obtained from each of the rodents collected during July and September and processed for spirochete isolation as described [20]. In addition, nymphal ticks removed from wood rats in July at the Fort Collins site were dissected and examined by darkfield and DFA microscopy [17] for the presence of spirochetes but were not cultured. The combined data from darkfield and DFA microscopy along with spirochete isolations were used to determine infection rates of host-associated nymphal and adult ticks.

Infection of xenodiagnostic ticks. A spirochete isolate from *Ixodes spinipalpis* (pool 1, 10 nymphs) was inoculated into two 3-week-old male ICR mice from the Centers for Disease Control and Prevention (CDC) colony maintained in Fort Collins. Mice were inoculated intraperitoneally or intradermally with ≥100,000 spirochetes contained in ≥0.1 mL of BSK-H. The primary isolate (passage 0) was used in these experiments. At 1 month after inoculation, ear tissue biopsy specimens were obtained from the mice as described [20]. Cultures were examined for spirochetes by darkfield microscopy until they were positive (≤1 week). Larval *I. scapularis* ticks from a spirochete-free colony derived from Great Island, Massachusetts, in 1985 [21] were allowed to feed to repletion on these mice at 5 weeks after inoculation. Replete larvae were held at 21°C and ≥90% relative humidity for >2 months. After molting, nymphs were examined for spirochetes by darkfield and DFA microscopy as described above. This experiment was done to determine whether a Colorado isolate could become established in a competent vector of Lyme disease.

Characterization of spirochetes. Cultures of spirochetes obtained from both ticks and rodents were determined to be *B. burgdorferi* sensu lato by a modification of our previously described polymerase chain reaction (PCR) method [22]. This nested-primer method detects *B. burgdorferi* by species-specific amplification of the flagellin (*fla*) gene. To reduce the risk of amplicon contamination, both amplifications were done in a single tube. The first reaction mixture (50 μL) contained 0.4 μ*M* outer primers 1 and 2 [22], 200 μ*M* each NTP, 3.25 m*M* $MgCl_2$, 5 μL of template in TRIS-EDTA buffer, and 2.5 units of Taq polymerase, all in 10 m*M* TRIS-HCl (pH 8.2) and 50 m*M* KCl buffer. This reaction mixture was overlaid with 100 μL of mineral oil and amplified for 40 cycles in a thermal cycler (System 9600; Perkin-Elmer Cetus, Norwalk, CT). Templates were initially denatured for 4 min at 95°C. Subsequent thermal cycles were 93.3°C for 24 s (denaturation), 55°C for 22 s (annealing), and 72°C for 54 s (extension). After the first amplification, a second reaction mixture (150 μL) containing 0.67 μ*M* inner primers 1 and 2 [23] and an additional 2.5 units of Taq polymerase was carefully added on top of the mineral oil. Buffer, salts, and nucleotides were at the same concentrations in the first and second reactions. The second reaction mixture was mixed with the first by centrifugation and amplified for 40 thermal cycles. The molar ratio of inner and outer primers was nominally 5:1 during the second amplification (neglecting the consumption of outer primers during the first 40 cycles).

Other contamination control measures, analysis of PCR products by *Pvu*II restriction enzyme digestion, and agarose gel electrophoresis were done as described [22].

PCR results were verified by amplifying a second target. We used a test that amplifies a genus-specific region of the *fla* gene [23]. These amplicons were also digested with *Pvu*II; the fragments were determined to be of appropriate size by electrophoresis in agarose.

Proteins for SDS-PAGE were prepared from 100-mL cultures of passage 2 spirochetes. Cultures were judged to be free of contaminating microorganisms when no growth was observed after 2 weeks of incubation in four standard bacteriologic media (brain-heart infusion broth and trypticase soy agar, 37°C and ambient temperature; thioglycollate broth, 37°C; and Sabouraud's agar, ambient temperature). Spirochetes were harvested by centrifugation and washed twice by suspending them in buffer supplemented with divalent cations (PBS plus 5 m*M* $MgCl_2$). Spirochetal proteins were adjusted to 1 mg/mL (BioRad, Richmond, CA; protein assay, bovine IgG standard) in loading buffer and electrophoresed at 40 mA by previously described methods [24]. Resolving gels (11 cm path length, 1.5 mm thick) were 12.5% *bis*-acrylamide overlaid by a 4% stacking gel (5 cm). Protein density was 10 μg/well.

Proteins were transferred from gels to nitrocellulose (0.2-μm pore size; Schleicher & Schuell, Keene, NH) by electroblotting (Semiphor TE 70 unit; Hoefer Scientific Instruments, San Francisco) at 100 mA for 1 h. Membranes were blocked overnight with 3% bovine serum albumin (fraction V) in 10 m*M* TRIS-HCl (pH 7.4) and 77 m*M* NaCl. Membranes were probed with the following antibodies: anti-OspA (outer surface protein A) monoclonal antibody (MAb) H5332 [25] (protein A–purified ascites, provided by R. A. Wirtz, Walter Reed Army Institute of Research, Washington, DC); anti-P39 MAb H1141 [26] and anti-OspC rabbit polyclonal antibody prepared against OspC from strain Sh-2-82 (provided by T. Schwan, NIH Rocky Mountain Laboratories, Hamilton, MT); and anti-flagellin MAb H9724 [27] (Symbicon, Umea, Sweden). Secondary antibodies were alkaline phosphatase–labeled goat anti-mouse IgM + IgG (heavy and light chain) or goat anti-rabbit IgG (heavy and light chain) (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted 1:1000 or 1:5000, respectively. Bound alkaline phosphatase was detected with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium in 0.1 *M* TRIS-HCl, pH 9.5.

Results

Collection of rodents and ticks. As summarized in table 1, 100 rodents and 944 ticks were collected during this study. Although the rate of capture varied between the three sites (Owl Canyon, 47% trap success; Fort Collins, 30%; and Carter Lake, 7%), *Neotoma mexicana* was the most frequently encountered rodent in all areas (88% of captured rodents). Other species collected were the bushy-tailed wood rat, *Neotoma cinerea*, the Colorado chipmunk, *Tamias quadrivittatus*, the rock squirrel, *Spermophilus variegatus*, and the rock mouse, *Peromyscus difficilis*.

I. spinipalpis was the principal tick parasitizing *N. mexicana* at the three sites (table 1). Of the other four species of rodents captured, only 1 *N. cinerea* at Owl Canyon had an adult *I. spinipalpis*, and 2 rock mice at Fort Collins had 7 larval *I. spinipalpis*. Populations of *I. spinipalpis* on *N. mexicana* were quite low at Owl Canyon (0.13 ticks/host) and Carter Lake (1.7 ticks/host) but considerably higher at Fort Collins (14.8 ticks/host). The only other tick species encountered during this study was *Dermacentor andersoni*, which represented <1% of all ticks collected (table 1).

Table 1. Numbers of rodents and ticks collected at three sites in Larimer County, Colorado.

Location, species of rodent (no.)	*Ixodes spinipalpis*			*Dermacentor andersoni*	
	Adults	Nymphs	Larvae	Nymphs	Larvae
Owl Canyon					
Neotoma mexicana (23)	2	1	0	1	0
Neotoma cinerea (2)	1	0	0	0	0
Tamias quadrivittatus (3)	0	0	0	0	0
Fort Collins					
N. mexicana (62)	61	184	674	3	3
Peromyscus difficilis (6)	0	0	7	0	2
Carter Lake					
N. mexicana (3)	1	2	2	0	0
Spermophilus variegatus (1)	0	0	0	0	0
Total (100 rodents)	65	187	683	4	5

Attempts to collect questing *I. spinipalpis* from vegetation and rock and soil substrates within 10 m of active wood rat dens were unsuccessful. Eight person-hours were spent dragging for ticks around four nest sites that were known from April trapping results to be inhabited by *I. spinipalpis*–infested *N. mexicana*. In contrast, 28 larvae (3, 7, 14, and 4) and 1 nymph were collected from inside the four nests by the flannel–flexible cable device. Because previous work has indicated that transovarial transmission of *B. burgdorferi* is rare in *Ixodes* ticks [28–30], larvae were not examined for spirochetes. The single nymph was negative for *B. burgdorferi* by darkfield and DFA microscopy.

Spirochete isolations. Table 2 summarizes the number of spirochetal isolates from *I. spinipalpis* taken from *N. mexicana* at two of the three study sites: 23 isolates were obtained from 87 ticks. The first 3 isolates originated from two pools of 10 nymphs each and one pool of 6 males that were collected in April. An additional 6 isolates were obtained from 4 of 11 individual nymphs and 2 of 4 individual adults. The first 9 isolates, collected at the Fort Collins site, were obtained from ticks triturated in tissue grinders after surface disinfection.

The remaining 14 tick-derived spirochetal isolates resulted from directly transferring aliquots of tick midgut–PBS suspension from microscope slides to BSK-H culture tubes. The 14 cultures positive for spirochetes were derived from 12 nymphs and 2 adults, all of which were positive by darkfield microscopy. Two positive ticks came from Carter Lake and 12 were collected at the Fort Collins site (table 2). This technique proved very successful and may be useful for spirochete isolation from other areas where implicated tick vec-

Table 2. Number of isolates of *Borrelia burgdorferi* from *Ixodes spinipalpis* collected from Mexican wood rats at three sites in Larimer County, Colorado.

Location, stage of *I. spinipalpis*	No. assayed	No. of isolates
Owl Canyon		
Adult	3	0
Nymph	1	0
Fort Collins		
Adult	12*	4
Nymph	68†	17
Carter Lake		
Adult	1	1
Nymph	2	1
Total	87	23

* Includes 6 individuals and pool of 6 adults; 3 individuals and pool of adults were positive for *B. burgdorferi*.

† Includes 48 individuals and two pools of 10 nymphs; 15 individuals and both pools of nymphs were positive for *B. burgdorferi*.

tors have not been identified. The 4 *I. spinipalpis* tested from the Owl Canyon site were not infected with spirochetes. None of the cultures originating from dissected tick midguts exhibited growth of bacterial cocontaminants.

Fifty-eight *I. spinipalpis* nymphs and 6 adults removed from *N. mexicana* at the Fort Collins site were tested for spirochetes by darkfield or DFA microscopy, culture, or a combination of methods; 38 of the nymphs (66%) were infected with spirochetes. Although the sample size of individual *I. spinipalpis* adults tested for spirochetes was small (6), half were infected with spirochetes. None of the 4 nymphal *D. andersoni* (table 1) collected from *N. mexicana* was infected with spirochetes.

As summarized in table 3, ear tissue biopsy samples were cultured for spirochetes from 83 rodents collected during July and September (17 *N. mexicana* collected in May were not tested), and 40 isolates were obtained. The results mirror those obtained from *I. spinipalpis* from the three areas surveyed. None of the 28 rodents from Owl Canyon was infected with spirochetes. Two of 3 *N. mexicana* from Carter Lake were positive for spirochetes. Thirty-six isolates were obtained from 45 wood rats (80%) collected at the Fort Collins site; 2 of 6 *P. difficilis* mice were infected with spirochetes.

Infection of xenodiagnostic ticks. Thirty replete larval *I. scapularis* ticks were recovered from each mouse infected with *I. spinipalpis*–derived spirochetes. At ≤1 month after they had molted to nymphs, 15 *I. scapularis* from each mouse were examined by darkfield and DFA microscopy. Twelve (40%) of 30 ticks were infected with spirochetes, demonstrating that spirochetes isolated from *I. spinipalpis* ticks were infective for mice and that these mice could, in turn, serve as sources of infection for ticks.

Characterization of spirochetes. DNA from cultured spirochetes was assayed by nested PCR that targeted a *B. burgdorferi* sensu lato–specific region of the *fla* gene. This *fla* region was amplified from all 63 spirochetal isolates. Amplicons from each isolate produced fragments of 247 and 143 bp when digested with *Pvu*II, as expected for this region of *fla*. Some of the isolates (36/63, 57%) were also tested with a single-amplification PCR that targeted a genus-specific region of *fla* [23]. All specimens tested were amplified and produced the expected *Pvu*II digestion products (not shown).

Of the 63 isolates of *B. burgdorferi*, 15 were chosen for antigenic studies: 9 from *I. spinipalpis*, 4 from *N. mexicana*, and 2 from *P. difficilis*. SDS-PAGE profiles and immunoblots of proteins from *B. burgdorferi* isolated from ticks and rodents are shown in figure 1. The isolates from individual ticks and rodents exhibited protein profiles characteristic of *B. burgdorferi* when compared with reference strains B31 and DN127 (figure 1 A, A′). Some diversity in apparent molecular mass of proteins was evident, particularly in the 21- to 35-kDa range. Expression of OspC was highly variable (brackets).

Immunoblotting studies provided further support for designating these spirochetes as *B. burgdorferi*. All isolates reacted with genus-specific anti-flagellin MAb H9724 (figure 1B, B′) and with the species-specific anti-OspA MAb H5332 (figure 1D, D′). The epitope recognized by anti-P39 MAb H1141 was expressed in some but not all isolates. This anti-P39 MAb reacted with 4 of 6 isolates from individual ticks, 3 of 3 from tick pools, and none from rodents (figure 1C, C′). A polyclonal antibody recognized OspC of variable apparent size in all isolates (figure 1E, E′); this variability (23 ± 1 kDa) is strikingly illustrated in lane 3, where two OspC bands are apparent in the lysate from a pool of *I. spinipalpis*.

Discussion

This study identifies a previously undescribed enzootic cycle of *B. burgdorferi* in the Rocky Mountain region of the

Table 3. Number of isolates of *Borrelia burgdorferi* from rodents collected at three sites in Larimer County, Colorado.

Location, rodent species	No. tested	No. positive (%)
Owl Canyon		
Neotoma mexicana	23	0
Neotoma cinerea	2	0
Tamias quadrivitattus	3	0
Fort Collins		
N. mexicana	45	36 (80)
Peromyscus difficilis	6	2 (33)
Carter Lake		
N. mexicana	3	2 (67)
Spermophilus variegatus	1	0
Total	83	40 (49)

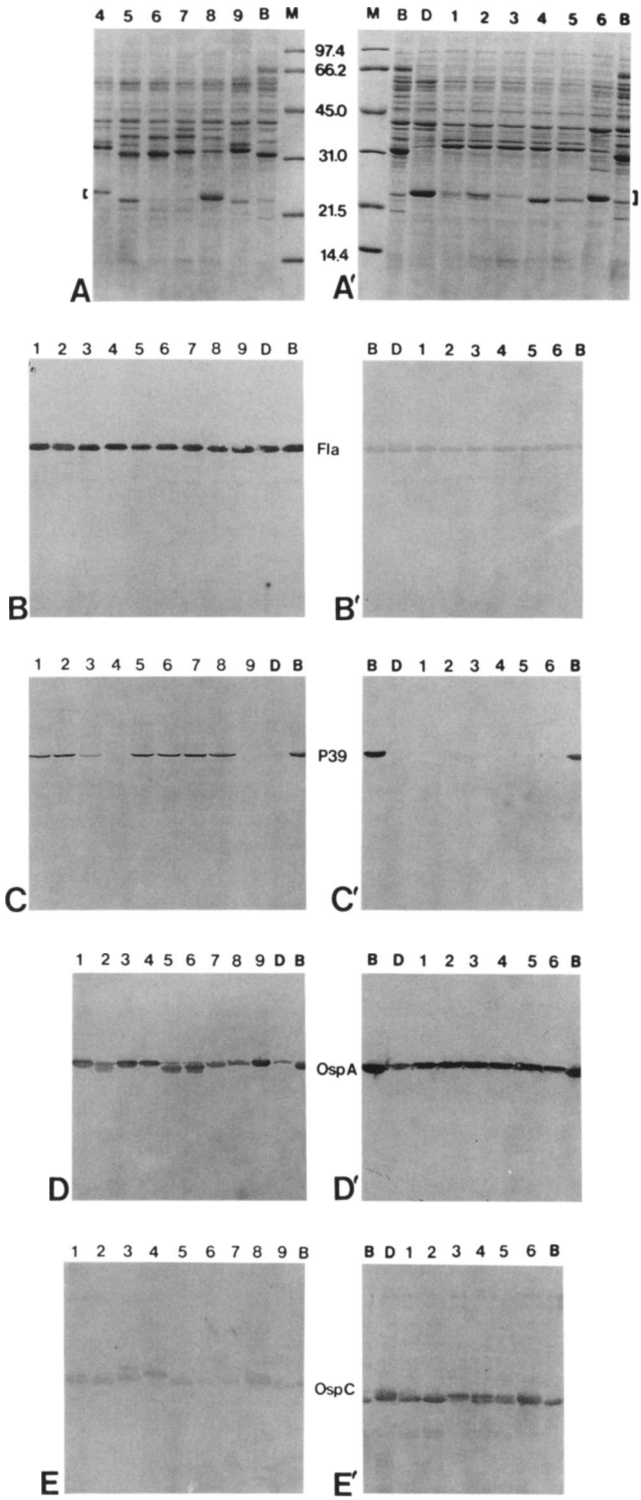

Figure 1. SDS-PAGE and immunoblot characterization of *B. burgdorferi* isolates from Colorado. **A–E**, spirochetes isolated from ticks (*I. spinipalpis*, IS): 1, IS1; 2, IS2; 3, IS4; 4, IS7; 5, IS11; 6, IS13; 7, IS14; 8, IS16; 9, IS19. **A′–E′**, spirochetes isolated from rodents (*N. mexicana*, NM; *P. difficilis*, PD): 1, NM47; 2, NM48; 3, NM54; 4, NM65; 5, PD 77; 6, PD81. B, D = reference strains B31 passage 9, DN127, respectively. M = molecular mass markers (kDa). **A**, **A′**: Protein profiles of *B. burgdorferi* lysates stained with Coomassie brilliant blue. Brackets = range of migration of outer surface protein (Osp) C. **B**, **B′–E**, **E′**: Immunoblots of *B. burgdorferi* lysates with anti-Fla monoclonal antibody (MAb) H9724, anti-P39 MAb H1141, anti-OspA MAb H5332, and anti-OspC polyclonal antibody, respectively.

western United States. Our study is the first record of *B. burgdorferi* isolates obtained from *I. spinipalpis* ticks. However, in northern California, *I. spinipalpis* parasitizing the dusky-footed wood rat has also been found to be infected with *B. burgdorferi* (Lane RS, personal communication). We also have described the first isolates of *B. burgdorferi* from Mexican wood rats, *N. mexicana*, and rock mice, *P. difficilis*. Other enzootic cycles involving non–*Ixodes ricinus* complex ticks have been identified in California [13] and the northeastern United States [31], but the enzootic cycle discovered in our study is especially interesting because this Colorado site is hundreds of kilometers from other known foci of infection in the Midwest or Pacific Coast states [2].

Because of this apparent geographic isolation from other established transmission cycles, we used several confirmatory methods to verify the identity of spirochete isolates. A representative group of 9 tick and 6 rodent isolates all reacted with the anti-OspA MAb H5332 in immunoblotting assays. Although there are numerous records of *B. burgdorferi* sensu lato strains that do not react with this MAb, it is presumed to be species-specific and has been used extensively to verify that isolates are *B. burgdorferi* sensu stricto [32]. The molecular masses of the OspA for the Colorado isolates were variable but within 1 kDa of the apparent mass of the OspA calculated for strain B31 (31 kDa). Variability in the size of OspA has been documented for a variety of other *B. burgdorferi* isolates from many geographic areas [33–37].

Each of the isolates tested also reacted with anti-OspC polyclonal antisera. OspC is present on *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* [38] (the latter two are *B. burgdorferi* sensu lato), and an OspC homolog has been identified in other *Borrelia* species [39, 40]. Reactivity of our Colorado isolates with the anti-P39 MAb H1141 was variable: 4 of the 6 tick isolates and none of the rodent isolates reacted positively by immunoblotting. Lack of reactivity with H1141 has been noted in several *B. burgdorferi* isolates from *I. pacificus* [26]. P39 has not been identified in other *Borrelia* species and is thought to be species-specific [41–43]. All isolates reacted with the genus-specific MAb to flagellin [27]. In addition, preliminary plasmid profile analyses (data not shown) also indicated that our Colorado isolates have a diverse array of plasmids (7–11 bands) typical of low-passage *B. burgdorferi* [44].

I. spinipalpis has been reported from the following western states: California, Colorado, Idaho, Montana, Nevada, Oregon, South Dakota, Texas, Utah, and Washington [14–16].

All three life stages infest a broad range of hosts, including several species of rodents, lagomorphs, and some ground-dwelling birds [14–16]. Records from Larimer County, Colorado, include the following tick hosts: Mexican woodrats, bushy-tailed woodrats (*N. cinerea*), deer mice (*P. maniculatus*), rock mice, chipmunks, and cottontail rabbits (*Sylvilagus audubonii* and *Sylvilagus nuttalli*) (CDC, unpublished data). The primary host of *I. spinipalpis* at the Larimer County study sites is the Mexican wood rat, which is probably also the major vertebrate reservoir of *B. burgdorferi* in the area. These results are similar to those reported from northern California, where enzootic cycles of *B. burgdorferi* have been described that involve *N. fuscipes* and *Ixodes neotomae* [13] and *N. fuscipes* and *I. spinipalpis* (Lane RS, personal communication). We also found *I. spinipalpis* ticks on the small number of rock mice examined. *B. burgdorferi* isolates were obtained from 2 of the 6 mice tested, suggesting that these rodents might also act as reservoir hosts for *B. burgdorferi* in Colorado. The distribution and habitat preference in Colorado of the rock mouse is identical to that of *N. mexicana* [45], and this mouse is often found nesting in wood rat dens.

The semiarid environment of the Rocky Mountain foothills in northern Colorado seems an unlikely site for an enzootic cycle of *B. burgdorferi*. This is especially true when one considers the high humidity requirements of *Ixodes* ticks [46]. This apparent paradox can be explained by the habitat preferences of *I. spinipalpis* ticks in Colorado. Although these ticks infest a variety of host species, off-host stages are found almost exclusively within wood rat nests. These nests provide a suitable microclimate, with high humidity and moderate temperatures, which enables these ticks to resist desiccation during parts of their life cycle when they are not attached and feeding on a host. The highly restricted distribution of the off-host stages of *I. spinipalpis* at our study site was demonstrated by our attempts to flag ticks in wood rat nests and areas surrounding these nests. We did not collect any ticks by flagging vegetation and ground surface substrates within 10 m of the nests; however, we did collect immature *I. spinipalpis* within wood rat nests by forcing the flannel–flexible cable device 1–2 m inside entrances to these nests.

The potential public health significance of these findings is unclear at present. Other than a single unpublished CDC record of an *I. spinipalpis* nymph taken from a child in northern New Mexico in 1991, we know of only one historical record: a female *I. spinipalpis* removed from a child in Linn County, Oregon, in 1942 [14]. Even if *I. spinipalpis* readily attacked humans when presented with the opportunity, the fact that questing ticks were found only inside wood rat nests at the Fort Collins study site suggests that humans are unlikely to be bitten by infected ticks in these areas. This may not be true, however, for *I. spinipalpis* populations in other regions of the western United States. For example, *I. spinipalpis* in a northern California study site were found not only in woodrat nests but also in the moist leaf litter surrounding these nests (Lane RS, personal communication). This observation is important because it suggests that humans or animals other than wood rats are more likely to come into contact with *I. spinipalpis* in northern California than in Colorado. Other areas in the West, including some sites in Colorado, also have habitats that are more mesic than our study areas. Such sites as riparian areas or montane forests and meadow edges near wood rat nesting habitats may have microclimates that allow *I. spinipalpis* to quest in areas away from wood rat nests or other protected sites.

Another notable difference between the public health implications of our results and those of the northern California studies [13] is that Colorado apparently lacks an *I. ricinus* complex tick comparable to *I. pacificus*, which is widespread in California. Unlike *I. spinipalpis*, *I. pacificus* not only is a competent vector of *B. burgdorferi* but also feeds on humans, thus acting as a "bridge vector" to transfer spirochetes from wood rat populations to humans [47].

Both *I. neotomae* and *I. spinipalpis* ticks and their wood rat hosts apparently support enzootic cycles of *B. burgdorferi* in California, but they probably only rarely are responsible for transmitting spirochetes to humans in that state. *I. pacificus* ticks, on the other hand, sometimes feed as larvae or nymphs on infected woodrats and then after molting to nymphs or adults, respectively, transmit *B. burgdorferi* to humans. Our xenodiagnosis experiment demonstrated that *B. burgdorferi* isolated from *I. spinipalpis* can infect *I. scapularis* and that, once infected, *I. scapularis* maintains these spirochetes transstadially. This indicates that other *I. ricinus* complex ticks could become infected with the *I. spinipalpis* spirochete and act as bridge vectors.

Such involvement of a second tick species to serve as a vector to humans is unlikely in Colorado, however, because the only ixodid tick that commonly attacks humans in the central Rocky Mountain states is *D. andersoni*. This tick has not been reported to be infected with *B. burgdorferi*, and its vector competence has yet to be evaluated, but the closely related species *Dermacentor variabilis* [48, 49] and *Dermacentor occidentalis* [50] are poor experimental vectors of *B. burgdorferi*.

Thus, evidence to date indicates that *B. burgdorferi* exists in northern Colorado, where it is maintained in an enzootic cycle involving *I. spinipalpis* ticks and their hosts. However, the restricted habitat requirements and host specificity of *I. spinipalpis* suggest that the risk of humans acquiring *B. burgdorferi* infection directly from *I. spinipalpis* is extremely low.

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