

Genetic Characteristics of *Borrelia coriacea* Isolates from the Soft Tick *Ornithodoros coriaceus* (Acari: Argasidae)

MAVIS HENDSON* AND ROBERT S. LANE

Division of Insect Biology, University of California, Berkeley, California 94720

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Two *Borrelia* isolates (CA434 and CA435) cultured from the soft tick *Ornithodoros coriaceus* were analyzed by contour-clamped homogeneous electric field gel electrophoresis of unrestricted and *ApaI*-restricted DNA, standard electrophoresis of *BamHI*- and *HindIII*-restricted DNA, Southern hybridization, restriction fragment length polymorphism and sequencing of the 16S rRNA gene, and amplification of the 5S-23S intergenic spacer region. These isolates were compared with *Borrelia coriacea* type strain Co53, *B. burgdorferi* sensu stricto strain CA4, and the relapsing-fever spirochete *B. parkeri* (undesigned). The 16S rRNA region of CA434 and CA435 differed from that of *B. coriacea* type strain Co53 by the presence of 1 base (C) at position 367 (GenBank accession no. U42286). The linear plasmid profile of CA434 was similar to that of Co53, and the *ApaI*, *BamHI*, and *HindIII* restriction fingerprints of the total cellular DNA of CA434 and Co53 were similar. In contrast, CA435 differed somewhat from CA434 and Co53, which demonstrates that *B. coriacea* is genetically diverse. Southern hybridization showed that the DNAs of CA434 and CA435 hybridized strongly with the digoxigenin-labeled DNA of Co53. Low homology was found between the DNA of Co53 and that of *B. parkeri*. The 16S rRNA sequence of *B. parkeri* was identical to previously published results for *B. parkeri* strain M3001 (GenBank accession number U42296). CA434 and CA435 represent only the second and third isolates of *B. coriacea* obtained from any source since its initial isolation from an *O. coriaceus* tick in 1985. All three *B. coriacea* isolates were derived from adult ticks collected from the same locality in northwestern California. Difficulties encountered in detecting *B. coriacea* in, and isolating this spirochete from, the tissues of *O. coriaceus* are discussed. The lack of concordance between different detection or isolation methods suggests that reliance upon a single technique may grossly underestimate the true prevalence of spirochetal infection in wild-caught *O. coriaceus* ticks.

In 1985, a previously unrecognized *Borrelia*-like spirochete was detected in all three trophic stages of the human-biting soft tick *Ornithodoros coriaceus* from northern California, and a single isolate from a male tick was partially characterized morphologically and immunochemically (15). Shortly thereafter, this isolate, designated Co53, was characterized genetically and phenotypically and named *Borrelia coriacea* after its tick vector (14, 19). *B. coriacea* was found to be passed transstadially and occasionally via eggs in its tick vector (15, 17), and the Columbian black-tailed deer (*Odocoileus hemionus columbianus*) was implicated as a probable natural host of the spirochete (16). Furthermore, circumstantial evidence suggested that *B. coriacea* is the cause of epizootic bovine abortion (EBA), a significant disease of rangeland cattle in some areas of the far-western United States, particularly California (15). Subsequent studies, while not ruling out the possibility that *B. coriacea* or another spirochete is involved in the epizootology of EBA, have not established a firm link between the presence of spirochetes and bovine abortion (28, 29, 37, 38, 41).

To our knowledge, no more isolates of *B. coriacea* have been isolated since 1985. Here we genetically characterize two more recently acquired isolates of *B. coriacea* that were derived from *O. coriaceus* ticks and compare them with the Co53 type strain of *B. coriacea*, the relapsing-fever spirochete *B. parkeri*, and the Lyme disease spirochete *B. burgdorferi* sensu stricto (s.s.). These new isolates of *B. coriacea*, designated CA434 and CA435, represent only the second and third iso-

lates of this little-known borrelial genospecies. As such, they provide an opportunity to evaluate the genetic variability inherent in *B. coriacea* from the same locality as strain Co53.

MATERIALS AND METHODS

Tick collection and dissection. Nymphal and adult *O. coriaceus* ticks were collected in enamelware pitfall traps baited with dry ice in chaparral and woodland-grass plant communities at the University of California Hopland Research and Extension Center (HREC) in Mendocino County, Calif., from July to September 1995. Ticks were surface disinfected for approximately 30 s each in 3% hydrogen peroxide and 70% ethanol and then rinsed in sterile phosphate-buffered saline. Next, they were embedded in paraffin and dissected in sterile phosphate-buffered saline and tissue smears were prepared from the central ganglion and portions of the midgut diverticula and salivary glands of each tick. After smears were air dried and fixed in acetone (10 min), they were examined for the presence of spirochetes by direct immunofluorescence (DI) assay with a fluorescein isothiocyanate-labeled rabbit anti-*B. burgdorferi* conjugate (4). The anti-*B. burgdorferi* conjugate was prepared with type strain B31 and purified on a protein A-Sepharose column (4).

Isolation procedures. After tissue smears had been prepared for the DI assay, the remaining tissues from each tick were subdivided and placed into two 1.5-ml Eppendorf tubes filled with 1.25 ml of BSK-II medium (2) containing rifampin at 25 µg/ml. One member of each pair of culture tubes was incubated at 30°C, the other was incubated at 36°C, and all cultures were examined for the presence of spirochetes weekly for 6 weeks by dark-field microscopy. Prior to genetic characterization, the borrelial isolates obtained were kept frozen at -74°C in a mixture of 3 parts glycerol and 7 parts BSK-II medium. Two of five borrelial isolates were regrown successfully in BSK-H medium (30) at 34.5°C, whereas attempts to regrow them in BSK-II medium were unsuccessful.

Isolates. The isolates compared were the type strain of *B. coriacea* (Co53) from the HREC, Mendocino County, Calif., two borrelial isolates obtained from *O. coriaceus* ticks from the HREC during the present study (CA434 and CA435), an isolate of the relapsing-fever spirochete *B. parkeri* (undesigned) from an *O. parkeri* tick, and the CA4 strain of *B. burgdorferi* s.s. from an *Ixodes pacificus* tick from Sonoma County, Calif. (18, 21). The *B. parkeri* isolate was obtained from the Rocky Mountain Laboratories Bacterial Collection through the courtesy of Tom Schwan, Hamilton, Mont. No information is available about the geographical origin of the latter isolate. The passage numbers for CA434, CA435, and

* Corresponding author. Present address: Department of Justice, 626 Bancroft Way, Berkeley, CA 94710. Phone: (510) 540-2434. Fax: (510) 540-2701. E-mail: hendsonm@hdcdojnet.state.ca.us.

TABLE 1. Primers used for PCR amplification and sequencing of the 16S rRNA region (26)

Primer	Sequence (5'-3')	Position (<i>E. coli</i> numbering)
fD3	AGAGTTTGATCCTGGCTTAG	8-27
T50	GTTACGACTTCACCCCTCT	1540-1522
400F	GGAGCGACACTGCGTG	414-429
500R	CTGCTGGCACGTAATTAGCC	548-529
800F	ATTAGATACCCTGGTAG	812-828
800R	CTACCAGGGTATCTAAT	828-812
1200F	TATGTCTGGGCTACACACG	1248-1267
1200R	CGTGTGTAGCCCAGGACATA	1267-1248

CA4 are six, six, and five, respectively. The passage numbers for *B. parkeri* and Co53 are unknown, but that of Co53 is greater than 10.

DNA extraction, restriction digestion, and agarose gel electrophoresis. Bacterial DNA was extracted using aerosol-resistant tips and following the cetyltrimethylammonium bromide miniprep method described by Wilson (39). Cetyltrimethylammonium bromide-purified DNAs were restricted separately with *Bam*HI and *Hind*III (New England Biolabs, Beverly, Mass.) in accordance with the manufacturer's instructions. Restriction DNA fragments were separated in a 1% TAE agarose gel (23) at 1.5 V/cm for 20 h. After electrophoresis, the gel was stained in a 0.5- μ g/ml ethidium bromide bath for 30 min. DNA was illuminated with UV light and photographed.

CHEF gel electrophoresis. DNA for contour-clamped homogeneous electric field (CHEF) gel electrophoresis was prepared by a modification of the method of Cooksey and Graham (5). Bacterial cells were washed with SE buffer (75 mM NaCl, 25 mM EDTA, pH 7.5), and the cell density was adjusted to $\sim 10^8$ CFU/ml in SE buffer. A 0.5-ml sample of the bacterial suspension was mixed with 0.5 ml of 2% agarose heated to 60°C (Pulsed Field Certified Agarose; Bio-Rad, Richmond, Calif.) in 10 mM Tris-HCl-10 mM MgCl₂-0.1 mM EDTA (pH 7.5). Plugs were cast in plug molds (1.0 by 0.5 cm; Bio-Rad) and held at 4°C for 5 min to allow the agarose to solidify. Ten plugs were placed in 5 ml of lysis solution consisting of proteinase K (0.5 mg/ml; Bethesda Research Laboratories, Gaithersburg, Md.), 1% *N*-lauroylsarcosine, and 0.5 M EDTA (pH 9.5). After overnight incubation at 50°C, the plugs were washed with gentle agitation four times for 1 h each time with 20 mM Tris-HCl (pH 8.0)-50 mM EDTA at room temperature. The plugs then were washed five times with TE (10 mM Tris, 10 mM EDTA, pH 8.0) at room temperature with a 10-min wash cycle. Prior to restriction digestion, the plugs were equilibrated in 1 \times restriction buffer A (Boehringer Mannheim, Indianapolis, Ind.) at room temperature for 1 h. DNA restrictions were carried out in 1 \times restriction buffer A-1.0 mg of bovine serum albumin per ml-10 U of *Apa*I (Boehringer Mannheim) overnight at 37°C. CHEF gel electrophoresis of undigested and *Apa*I-restricted DNA was carried out in a 1% agarose gel (13 by 14 cm) prepared with 0.5 \times TBE buffer (23). Electrophoresis of *Apa*I-digested DNA was carried out in 0.5 \times TBE at 14°C for 18 h at 6 V/cm with a 1- to 30-s switch time ramp at an included angle of 120° using a CHEF-DRIII pulsed-field electrophoresis system (Bio-Rad). Electrophoretic conditions for undigested DNA were the same, except that the switch time ramp was 0.5 to 3.5 s for 16 h. Agarose plugs containing concatemers of lambda DNA (Bio-Rad) were used as molecular weight standards. The gel was stained and photographed as described above.

Southern blotting and hybridization. Restricted fragments were transferred by capillary action to Magnagraph nylon membranes (MSI, Westborough, Mass.) by Southern blotting as described previously (23). After transfer, DNA was UV cross linked to the membrane using a Stratilinker (Stratagene, La Jolla, Calif.). The probe was prepared by restricting total DNA of strain Co53 with *Hind*III and labeling the fragments with digoxigenin using the Genius DNA labeling kit as described by the manufacturer (Boehringer Mannheim). Southern hybridization and detection of DNA which hybridized to the probe was carried out using the Genius detection kit (Boehringer Mannheim).

Amplification and restriction polymorphism of the 16S rRNA region. The 16S rRNA region of all isolates was amplified using primers fD3 and T50 (26). The DNA Thermal Cycler (Perkin-Elmer) was used with 1 cycle of 1 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C and a final extension cycle of 10 min at 72°C.

Ten microliters of the amplification products was restricted separately with *Bfa*I, *Hph*I, and *Mse*I (New England Biolabs, Beverly, Mass.). Restriction fragments were resolved in a 2.5% NuSieve GTG agarose gel (FMC BioProducts, Rockland, Maine) with 1 \times TBE (23) at 5 V/cm for 3.5 h.

Sequencing of the 16S rRNA region. The 16S rRNA amplification products of all isolates were purified using Millipore ultrafree tubes (Millipore, Bedford, Mass.). Both DNA strands were sequenced by cycle sequencing using the Terminator Ready Reaction Kit (Perkin-Elmer/Applied Biosystems, Foster City, Calif.) as recommended by the manufacturer. Primers fD3, 400F, 500R, 800F, 800R, 1200F, 1200R, and T50 (26) (Table 1) were used for sequencing. Amplification using primers 800F and 800R was carried out with 1 cycle of 96°C for 2

TABLE 2. Distribution of *B. coriacea* in tissues^a of nymphal and adult *O. coriaceus* ticks and results of isolation attempts^b at two temperatures

Specimen no. and stage ^c	Spirochetal infection			Result of isolation attempt at:		Isolate no.
	Central ganglion	Midgut	Salivary gland	30°C	36°C	
1F	-	-	-	+	+	CA432
2M	-	-	-	-	+	CA433
3F	-	-	-	+	+	CA434
4M	-	-	-	-	+	CA435
5F	+	+	+	+	-	CA436
6M	-	-	-	+	-	- ^d
7M	+	-	-	-	-	-
8M	-	+	-	-	-	-
9N	+	-	+	-	-	-

^a Determined by DI examination of tissue smears.

^b In BSK-II medium.

^c N, nymph; F, female; M, male.

^d Isolate lost before culture stocks could be prepared.

min and 25 cycles of 96°C for 30s, 48°C for 30s, and 60°C for 4 min. For primers fD3, 400F, 500R, 1200F, 1200R, and T50, the cycling parameters were the same except that an annealing temperature of 52°C was used. Sequencing products were electrophoresed and scanned using an ABI 377 automated sequencer (Perkin-Elmer/Applied Biosystems). Sequencher software (Version 3.0; Gene Codes Corp., Ann Arbor, Mich.) was used to align and analyze the DNA sequences.

Amplification for the specific detection of relapsing-fever borreliae and of the *rrf* (5S)-*rrl* (23S) intergenic spacer region. Primers REC4 and REC9 were used as described by Marti Ras et al. (26) for the identification of relapsing-fever borrelial isolates and *B. coriacea*. To determine whether isolates carried tandemly duplicated 5S (*rrf*)-23S (*rrl*) rRNA regions as found among strains of *B. burgdorferi* sensu lato (s.l.), amplification of the *rrf-rrl* intergenic region was carried out as described previously (31).

Nucleotide sequence accession numbers. The sequences of isolates CA434, CA435, Co53, *B. parkeri*, and CA4 have been submitted to GenBank. Their accession numbers are AF210134 to AF210138, respectively.

RESULTS

Tick studies. A total of 193 nymphs and adults of *O. coriaceous* were collected from chaparral and woodland-grass plant communities between 26 July and 22 September 1995. Seventy-one late-instar nymphs or adults were examined for the presence of spirochetes by DI and culture techniques. Spirochetes were detected, isolated, or both in 1 (3.0%) of 33 nymphs, 3 (18.8%) of 16 females, and 5 (22.7%) of 22 males. Spirochetes were detected in only four of the nine positive ticks by DI, two of which had disseminated infections involving two or three tissue types (Table 2). In contrast, spirochetes were isolated in BSK-II medium from six ticks at 30 or 36°C or at both temperatures. An isolate from a male tick (no. 6) was lost during cultivation and before aliquots could be frozen. Three of the four DI assay-positive ticks (no. 7 to 9) did not yield borrelial isolates at either temperature, and both isolates characterized in the present study were DI test negative (Table 2).

Attempts to regrow the five remaining isolates from the frozen stock cultures about 2.5 years later in order to characterize them met with partial success. Two isolates (CA434 and CA435) were successfully recultured in BSK-H medium but not in BSK-II medium.

Agarose gel electrophoresis of total DNA and Southern hybridization. CHEF gel electrophoresis of undigested DNA revealed a number of linear plasmids in all of the isolates (Fig. 1). Except for two linear plasmid bands that were absent from Co53, the plasmid profiles of CA434 and Co53 were identical

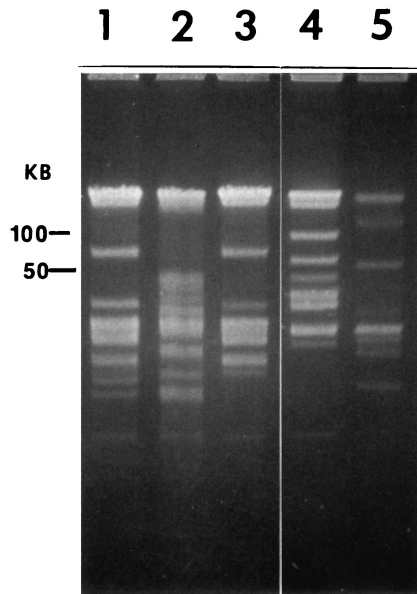


FIG. 1. CHEF gel electrophoresis of undigested DNAs of *Borrelia* isolates. Lanes: 1, CA434; 2, CA435; 3, Co53; 4, *B. parkeri*; 5, CA4. Linear plasmid DNA was resolved in 1% agarose-0.5× TBE (23) at 14°C for 16 h at 6 V/cm with a 0.5- to 3.5-s switch time ramp using a CHEF-DRIII pulsed-field electrophoresis system.

(Fig. 1, lanes 1 and 3). All of the other isolates (CA435, *B. parkeri*, and CA4) had unique plasmid profiles. Gel electrophoresis of *Bam*HI-, *Hind*III-, and *Apa*I-digested (results not shown) DNA indicated that all of the isolates had unique restriction fingerprints, with the exception of CA434 and Co53, which were similar.

Southern hybridization using Co53 total DNA as a probe demonstrated that the DNAs of CA434 and CA435 shared strong homology with the DNA of Co53. A low degree of homology was observed with *B. parkeri*, as indicated by fainter bands (Fig. 2), and no homology was observed with CA4.

Restriction fragment length polymorphism analysis of the 16S rRNA region. The 16S rRNA regions of isolates restricted with *Bfa*I, *Hph*I, and *Mse*I are shown in Fig. 3. Isolates CA434, CA435, and Co53 had identical *Bfa*I, *Hph*I, and *Mse*I restriction patterns, and they were identical to that previously reported for strain Co53 (26). The restriction patterns of isolate *B. parkeri* and *B. parkeri* strain M3001 were identical (26). The *Bfa*I restriction pattern of *B. parkeri* (undesigned) resembled that of CA4.

Sequence analysis of the 16S rRNA gene. Approximately 1,450 bases of the 16S rRNA gene of all isolates were sequenced. The sequences of isolates CA434, CA435, and Co53 were identical. These sequences were found to differ slightly from the previously reported 16S rRNA sequence of *B. coriaceae* Co53 (GenBank accession number U42286) by the addition of one base (cytosine) at position 367. The sequence of *B. parkeri* was identical to that of *B. parkeri* strain M3001 (GenBank accession number U42296).

Amplification of a region specific to relapsing-fever spirochetes and the *rrf*(5S)-*rrl*(23S) intergenic spacer region. Primers REC4 and REC9 (26) were used to amplify a region in the 16S rRNA gene specific to relapsing-fever spirochetes. A 523-bp amplification product was synthesized from the genomic DNAs of isolates CA434, CA435, Co53, and *B. parkeri* (Fig. 4). No amplification product was present for isolate CA4.

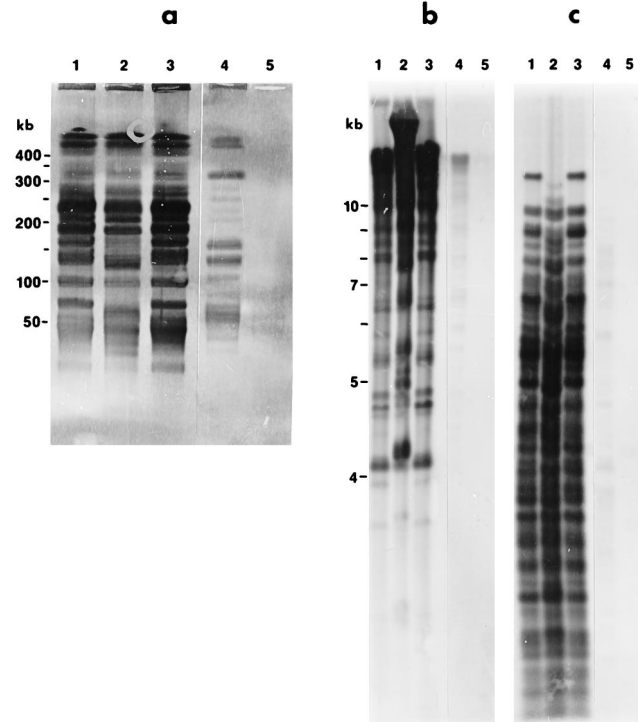


FIG. 2. Southern hybridization of *Apa*I (a)-, *Bam*HI (b)-, and *Hind*III (c)-digested DNAs of *Borrelia* isolates with digoxigenin-labeled Co53 DNA as a probe. Lanes: 1, CA434; 2, CA435; 3, Co53; 4, *B. parkeri*; 5, CA4. Southern hybridization and detection of DNA which hybridized to the probe were done as recommended by Boehringer Mannheim for the digoxigenin DNA labeling and detection kit.

An amplification product of approximately 250 bp was obtained for isolate CA4 using *rrf-rrl* primers 1 and 2 (31), whereas no amplification products were detected for isolates CA434, CA435, Co53, or *B. parkeri* (data not shown).

DISCUSSION

Molecular characteristics identify isolates CA434 and CA435 from the soft tick *O. coriaceus* as *B. coriaceae*. This is based on the 99.99% homology between the 16S rRNA gene

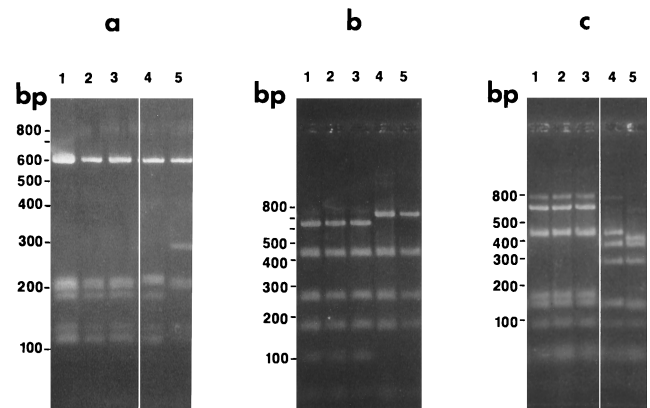


FIG. 3. 16S rRNA amplification products restricted with *Hph*I (a), *Bfa*I (b), and *Mse*I (c). Lanes: 1, CA434; 2, CA435; 3, Co53; 4, *B. parkeri*; 5, CA4. Restriction fragments were resolved in 2.5% NuSieve-GTG agarose-1× TBE at 5 V/cm for 3.5 h.

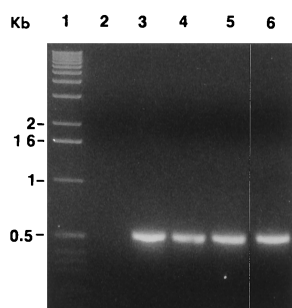


FIG. 4. Amplification products of *Borrelia* isolates using primers REC4 and REC9. Lanes: 1, molecular size marker ladder; 2, CA4; 3, CA434; 4, CA435; 5, Co53; 6, *B. parkeri*. Amplification products were electrophoresed in 1.4% agarose-1× TAE at 5 V/cm for 3 h.

sequences of CA434 and CA435 and that of the Co53 type strain of *B. coriaceae* (GenBank accession number U42286) (26). The use of the 16S rRNA gene locus to identify and confirm various bacterial groups, including the genus *Borrelia*, is well documented (1, 24, 40). Southern hybridization showed that Co53, CA434, and CA435 share DNA homology. Moreover, an amplification product was synthesized from the genomic DNAs of isolates CA434, CA435, and Co53 in PCRs with the REC4 and REC9 primers, the sequences of which are unique to the relapsing-fever spirochetes and *B. coriaceae* (26). An *rrf-rrl* (5S-23S rRNA) intergenic spacer region was absent from isolates CA434, CA435, and Co53, which indicates the presence of only one copy of the 5S-23S rRNA gene. In this respect, isolates CA434 and CA435 differ from the various genospecies of *B. burgdorferi* s.l., which possess a unique arrangement of ribosomal genes with one copy of the 16S (*rrs*) rRNA gene and two copies each of the 5S (*rrf*)-23S (*rrl*) rRNA genes (36). This kind of organization is not found in other members of the genus *Borrelia* or in other eubacteria (7).

The *Apa*I, *Bam*HI, and *Hind*III restriction digestion patterns of the total DNAs of isolates Co53 and CA434 were similar but distinct from that of CA435. CHEF electrophoresis of undigested linear plasmid DNA (6) revealed that isolate CA434 carried the same-size linear plasmids as Co53, except that two plasmids were absent from Co53. It is possible that the two plasmids missing in Co53 were lost during prolonged laboratory cultivation, which occurs commonly among *B. burgdorferi* isolates subjected to many laboratory passages (35).

The fact that isolate CA435 differs from Co53 and CA434 indicates that genetic variability exists among *B. coriaceae* strains. In this regard only, *B. coriaceae* resembles *B. burgdorferi* s.l., in which considerable genetic heterogeneity has been observed with various techniques (20, 27, 34, 35). Further, our findings concur with those of LeFebvre and Perng (19), who found that Co53 shares very little DNA homology with genomes of the *B. burgdorferi* s.l. complex, which is not unexpected because of the similarity of Co53 to the relapsing-fever spirochetes (26). We compared all three known *B. coriaceae* isolates with the relapsing-fever spirochete *B. parkeri* because of their genetic relatedness (26) and with the Lyme disease spirochete *B. burgdorferi* s.s. because the geographical distributions of their primary tick vectors overlap in California (10).

CA434 and CA435 represent only the second and third isolates of *B. coriaceae* since this spirochete was first isolated and characterized over a decade ago (14, 15). Both isolates were obtained from the same species of human-biting soft tick and at the same locality in northwestern California as strain Co53. Repeated attempts to cultivate *B. coriaceae*-like spirochetes

from DI test-positive *O. coriaceus* ticks collected in the Hopland area since the mid-1980s were unsuccessful (R. S. Lane, unpublished data) until 1995. The fact that only two of five borrelial isolates obtained from *O. coriaceus* ticks in 1995 could be regrown less than 3 years later from frozen stock cultures, and then only in BSK-H medium, exemplifies the difficulties encountered in culturing this organism. Our inability to routinely cultivate *B. coriaceae* from the tissues of spirochete-infected *O. coriaceus* ticks in BSK-II medium suggests either that this spirochete is a more fastidious organism than *B. burgdorferi* or that it has different maintenance and growth requirements.

Two methods, DI assay and culture, were used to detect spirochetes in *O. coriaceus* tissues, but the concordance between these methods was poor (Table 2). In fact, only one of six ticks that initially yielded an isolate was also DI test positive and, conversely, just one of four DI test-positive ticks produced an isolate. Furthermore, spirochetes from four of the six original isolates cultivated in BSK-II medium grew at either 30 or 36°C but not at both temperatures. In striking contrast, the culture-adapted B-31 type strain of *B. burgdorferi* s.s. grows well, albeit at variable rates, in BSK-H medium at temperatures ranging between 25 and 37°C (12). We tried to cultivate spirochetes from tick tissues at both 30 and 36°C in the present study because of the minimal previous success achieved in growing *B. coriaceae* from DI test-positive ticks in BSK-II medium at ~35°C (15, Lane, unpublished data). Our data demonstrate the value of using more than one method to detect spirochetes when determining infection prevalences in tick populations. They also suggest that reliance upon a single detection method or the use of a single temperature regimen or type of liquid growth medium during efforts to isolate *B. coriaceae* may result in a gross underestimate of the true infection prevalence.

Despite the fact that *B. coriaceae* was known from a single isolate before the present study, researchers have investigated many aspects of the biology of this poorly known spirochete in relation to other spirochetes, including *B. burgdorferi*. For example, its growth in BSK-H culture medium (30), phylogenetic relatedness to other *Borrelia* spp. (8, 9, 26), proteins (3, 11, 13, 25, 32, 33), and enzyme activity (22) have been studied just since 1993. *B. coriaceae* spirochetes also have been shown to be of low infectivity for infant Swiss White mice, infant BALB/c mice, and adult New Zealand White rabbits (15). The availability of two additional low-passage isolates of *B. coriaceae* provides the scientific community with an opportunity to expand these studies and perhaps to determine definitively whether *B. coriaceae* is somehow involved in the etiology of EBA in the far-western United States.

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REFERENCES

- Adam, T., B. Graf, U. Neubert, and U. B. Gobel. 1992. Detection and classification of *Borrelia burgdorferi* by direct sequencing of 16S rRNA amplified after reverse transcription. *Med. Microbiol. Lett.* 1:120-126.
- Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.* 57:521-525.
- Bunikis, J., C. J. Luke, E. Bunikiene, S. Bergstrom, and A. G. Barbour. 1998.

- A surface-exposed region of a novel outer membrane protein (P66) of *Borrelia* spp. is variable in size and sequence. *J. Bacteriol.* **180**:1618–1623.
4. Burgdorfer, W., R. S. Lane, A. G. Barbour, R. A. Gresbrink, and J. R. Anderson. 1985. The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. *Am. J. Trop. Med. Hyg.* **34**:925–930.
 5. Cooksey, D. A., and J. H. Graham. 1989. Genomic fingerprinting of two pathovars of phytopathogenic bacteria by rare-cutting restriction enzymes and field inversion gel electrophoresis. *Phytopathology* **79**:745–750.
 6. Ferdows, M. S., and A. G. Barbour. 1989. Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent. *Proc. Natl. Acad. Sci. USA* **86**:5969–5973.
 7. Fukunaga, M., and M. Sohnaka. 1992. Tandem repeat of the 23S and 5S ribosomal RNA genes in *Borrelia burgdorferi*, the etiological agent of Lyme disease. *Biochem. Biophys. Res. Commun.* **183**:952–957.
 8. Fukunaga, M., Y. Takahashi, Y. Tsuruta, O. Matsushita, D. Ralph, M. McClelland, and M. Nakao. 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *Int. J. Syst. Bacteriol.* **45**:804–810.
 9. Fukunaga, M., K. Okada, M. Nakao, T. Konishi, and Y. Sato. 1996. Phylogenetic analysis of *Borrelia* species based on flagellin gene sequences and its application for molecular typing of Lyme disease borreliae. *Int. J. Syst. Bacteriol.* **46**:898–905.
 10. Furman, D. P., and E. C. Loomis. 1984. The ticks of California (Acari: Ixodida). *Bull. Calif. Insect Surv.* **25**:1–239.
 11. Gilmore, R. D., Jr., K. J. Kappel, and B. J. B. Johnson. 1997. Molecular characterization of a 35-kilodalton protein of *Borrelia burgdorferi*, an antigen of diagnostic importance in early Lyme disease. *J. Clin. Microbiol.* **35**:86–91.
 12. Heroldova, M., M. Nemecek, and Z. Hubalek. 1998. Growth parameters of *Borrelia burgdorferi* sensu stricto at various temperatures. *Zentbl. Bakteriol.* **288**:451–455.
 13. Hu, L. T., S. D. Pratt, G. Perides, L. Katz, R. A. Rogers, and M. S. Klempner. 1997. Isolation, cloning, and expression of a 70-kilodalton plasminogen binding protein of *Borrelia burgdorferi*. *Infect. Immun.* **65**:4989–4995.
 14. Johnson, R. C., W. Burgdorfer, R. S. Lane, A. G. Barbour, S. F. Hayes, and F. W. Hyde. 1987. *Borrelia coriaceae* sp. nov.: putative agent of epizootic bovine abortion. *Int. J. Syst. Bacteriol.* **37**:72–74.
 15. Lane, R. S., W. Burgdorfer, S. F. Hayes, and A. G. Barbour. 1985. Isolation of a spirochete from the soft tick, *Ornithodoros coriaceus*: a possible agent of epizootic bovine abortion. *Science* **230**:85–87.
 16. Lane, R. S., and W. Burgdorfer. 1988. Spirochetes in mammals and ticks (Acari: Ixodidae) from a focus of Lyme borreliosis in California. *J. Wildl. Dis.* **24**:1–9.
 17. Lane, R. S., and S. A. Manweiler. 1988. *Borrelia coriaceae* in its tick vector, *Ornithodoros coriaceus* (Acari: Argasidae), with emphasis on transstadial and transovarial infection. *J. Med. Entomol.* **25**:172–177.
 18. Lane, R. S., and J. A. Pascocello. 1989. Antigenic characteristics of *Borrelia burgdorferi* isolates from ixodid ticks in California. *J. Clin. Microbiol.* **27**:2344–2349.
 19. LeFebvre, R. B., and G.-C. Perng. 1989. Genetic and antigenic characterization of *Borrelia coriaceae*, putative agent of epizootic bovine abortion. *J. Clin. Microbiol.* **27**:389–393.
 20. LeFebvre, R. B., G.-C. Perng, and R. C. Johnson. 1989. Characterization of *Borrelia burgdorferi* isolates by restriction endonuclease analysis and DNA hybridization. *J. Clin. Microbiol.* **27**:636–639.
 21. LeFebvre, R. B., R. S. Lane, G.-C. Perng, J. A. Brown, and R. C. Johnson. 1990. DNA and protein analyses of tick-derived isolates of *Borrelia burgdorferi* from California. *J. Clin. Microbiol.* **28**:700–707.
 22. Manafi, M., and G. Stanek. 1994. Enzyme activities of Lyme disease and relapsing fever borreliae. *Lett. Appl. Microbiol.* **19**:149–152.
 23. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 24. Marconi, R. T., and C. F. Garon. 1992. Identification of a third genomic group of *Borrelia burgdorferi* through signature nucleotide analysis and 16S rRNA sequence determination. *J. Gen. Microbiol.* **138**:533–536.
 25. Marconi, R. T., D. S. Samuels, T. G. Schwan, and C. F. Garon. 1993. Identification of a protein in several *Borrelia* species which is related to OspC of the Lyme disease spirochetes. *J. Clin. Microbiol.* **31**:2577–2583.
 26. Marti Ras, N., B. Lascola, D. Postic, S. J. Cutler, F. Rodhain, G. Baranton, and D. Raoult. 1996. Phylogenesis of relapsing fever *Borrelia* spp. *Int. J. Syst. Bacteriol.* **46**:859–865.
 27. Mathiesen, D. A., J. H. Oliver, Jr., C. P. Kolbert, E. D. Tullson, B. J. B. Johnson, G. L. Campbell, P. D. Mitchell, K. D. Reed, S. R. Telford III, J. F. Anderson, R. S. Lane, and D. H. Persing. 1997. Genetic heterogeneity of *Borrelia burgdorferi* in the United States. *J. Infect. Dis.* **175**:98–107.
 28. Osebold, J. W., R. Spezialetti, M. B. Jennings, R. F. Pritchett, and R. B. Bushnell. 1986. Congenital spirochetosis in calves: association with epizootic bovine abortion. *J. Am. Vet. Med. Assoc.* **188**:371–376.
 29. Osebold, J. W., B. I. Osburn, R. Spezialetti, R. B. Bushnell, and J. L. Stott. 1987. Histopathologic changes in bovine fetuses after repeated reintroduction of a spirochete-like agent into pregnant heifers: association with epizootic bovine abortion. *Am. J. Vet. Res.* **48**:627–633.
 30. Pollack, R. J., S. R. Telford III, and A. Spielman. 1993. Standardization of medium for culturing Lyme disease spirochetes. *J. Clin. Microbiol.* **31**:1251–1255.
 31. Postic, D., M. V. Assous, P. A. D. Grimont, and G. Baranton. 1994. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of *rfl* (5S)-*rrl* (23S) intergenic spacer amplicons. *Int. J. Syst. Bacteriol.* **44**:743–752.
 32. Sambri, V., F. Massaria, M. Ardizzoni, C. Stefanelli, and R. Cevenini. 1993. Glycoprotein patterns in *Borrelia* spp. *Zentbl. Bakteriol.* **279**:330–335.
 33. Sambri, V., C. Stefanelli, C. Rossoni, M. La Placa, and R. Cevenini. 1993. Acylated proteins in *Borrelia hermsii*, *Borrelia parkeri*, *Borrelia anserina*, and *Borrelia coriaceae*. *Appl. Environ. Microbiol.* **59**:3938–3940.
 34. Schmid, G. P., A. G. Steigerwalt, S. E. Johnson, A. G. Barbour, A. C. Steere, I. M. Robinson, and D. J. Brenner. 1984. DNA characterization of the spirochete that causes Lyme disease. *J. Clin. Microbiol.* **20**:155–158.
 35. Schwan, T. G., W. Burgdorfer, and C. F. Garon. 1988. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. *Infect. Immun.* **56**:1831–1836.
 36. Schwartz, J. J., A. Gazumyan, and I. Schwartz. 1992. rRNA gene organization in the Lyme disease spirochete, *Borrelia burgdorferi*. *J. Bacteriol.* **174**:3757–3765.
 37. Spezialetti, R., and J. W. Osebold. 1989. Lymphocyte blastogenesis and cellular cytotoxicity in a congenital infection of bovine fetuses related to epizootic bovine abortion. *Res. Vet. Sci.* **46**:160–167.
 38. Spezialetti, R., and J. W. Osebold. 1991. Surface markers on bovine fetal lymphocytes and immunoglobulin synthesis in a congenital infection related to epizootic bovine abortion. *Res. Vet. Sci.* **51**:239–245.
 39. Wilson, K. 1987. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.2. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (ed.), *Current protocols in molecular biology*. Greene Publishing Associates & Wiley-Interscience, New York, N.Y.
 40. Woese, C. R. 1987. Bacterial evolution. *Microbiol. Rev.* **51**:221–271.
 41. Zingg, B. C., and R. B. LeFebvre. 1994. Polymerase chain reaction for detection of *Borrelia coriaceae*, putative agent of epizootic bovine abortion. *Am. J. Vet. Res.* **55**:1509–1515.