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Genetic and Antigenic Characterization of *Borrelia coriaceae*, Putative Agent of Epizootic Bovine Abortion

RANCE B. LEFEBVRE* AND GUEY-CHUEN PERNG

Department of Veterinary Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California 95616

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Borrelia coriaceae **was characterized genetically and antigenically by utilizing the following techniques: restriction endonuclease analysis, Southern blotting and genomic hybridization, pulsed-field electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and immunoblotting. The *B. coriaceae* genome revealed unique and characteristic banding patterns both by agarose gel electrophoresis and by hybridization when compared with several *Borrelia burgdorferi* isolates. Pulsed-field gel electrophoresis demonstrated several linear plasmids ranging from 65 to 30 kilobase pairs. Cross-reaction with *B. burgdorferi* antigens ranging from 21 to 26 kilodaltons were demonstrated by immunoblotting with rabbit anti-*B. coriaceae* antiserum. However, most *B. coriaceae* antigens were quite distinct when compared with *B. burgdorferi* and *Leptospira interrogans* antigens.**

Borrelia coriaceae was isolated from the soft-bodied tick *Ornithodoros coriaceus* in 1985 (16). It was classified as a new species of *Borrelia* in 1987 (12). It has been described as the putative agent of epizootic bovine abortion (12, 16, 20, 21), although conclusive evidence of the role of the spirochete as a pathogen is still lacking. However, congenital spirochetosis in calves has been reported (21), in which the spirochete was observed in the blood of fetuses with lesions typical of epizootic bovine abortion. A highly motile, helical spirochete with morphologic features similar to those seen in infected calves was isolated from the soft-bodied tick as mentioned above (16). A monoclonal antibody directed against the periplasmic flagella of *Borrelia burgdorferi* reacted with *B. coriaceae* (12).

Previous work by Hyde and Johnson (11) demonstrated the relationship of *B. coriaceae* to several other *Borrelia* species at the genus level. The presence of a plasmid in *B. coriaceae* was also reported by them in the same manuscript.

In this report we further characterize *B. coriaceae* at the genetic and antigenic level by comparing it with several *B. burgdorferi* and *Leptospira interrogans* isolates. Restriction endonuclease analysis, Southern blotting (27), pulsed-field electrophoresis (26), and Western blotting (immunoblotting) were utilized to increase our understanding of this organism.

MATERIALS AND METHODS

Organisms. The *Borrelia* organisms used in this study were obtained from Russell Johnson, University of Minnesota. They were isolated from ticks, humans, and animals (Table 1) and were maintained in modified Barbour-Stoenner-Kelly (BSK II) medium (1) at 31°C.

The *L. interrogans* isolates used in this study were obtained from the National Leptospirosis Reference Center in Ames, Iowa. They were maintained in EMJH medium (Difco Laboratories, Detroit, Mich.) at 29°C.

Isolation and characterization of DNA. Whole-cell DNA was isolated by the procedure of LeFebvre et al. (17). Restriction endonuclease analysis was performed as previously described (29) and according to the manufacturers' specifications. The restriction fragments were separated by gel electrophoresis in a 0.7% agarose gel at 60 V for 15.5 h. The gel was then stained with ethidium bromide, illuminated with UV irradiation, and photographed. The DNA in the gel was then transferred to a nylon membrane by the method of Southern (27). Reference strain ATCC 43381 *B. coriaceae* was digested with the same restriction enzyme, radiolabeled with [α-^{32}P]dCTP by nick translation (24), and used as the probe for the blot. The hybridization and subsequent washes were carried out under stringent conditions (19). Briefly, the filters were washed in 2× SSC (from a 20× stock of 3 M sodium chloride and 0.3 M sodium citrate [pH 7.0]) and 0.5% sodium dodecyl sulfate and incubated at 68°C for 2 h. The buffer was then changed, and filters were incubated for an additional 30 min. The blot was then dried and exposed to X-ray film.

Pulsed-field gel electrophoresis. Samples for pulsed-field gel electrophoresis were prepared by the encapsulation method of Overhauser and Radic (22). Lambda phage DNA concatemers used as molecular weight markers (Stratagene Cloning Systems, San Diego, Calif.) were prepared as previously described (22, 31). Incert agarose (FMC BioProducts, Rockland, Maine) was used to prepare the samples. Approximately 3 μg of λ DNA, *B. burgdorferi*, and *B. coriaceae* samples were added to each well of a 1% agarose gel. Electrophoresis was performed in a Pharmacia LKB Pulsaphor System (Pharmacia Uppsala, Sweden) with 10-s switching time for 44 h at a constant voltage (330 V). The electrodes for south and east were set at 30°, 120°, and 210°. The electrodes for north and west were set at 60°.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. Discontinuous sodium dodecyl sulfate-gel electrophoresis was performed by the method of Laemmli (14) in 12% polyacrylamide gels. Whole-cell lysates from approximately 20 μg were added to each lane. Lysis buffer and running dye concentrations were prepared according to Pharmacia specifications. High- and low-molecular-weight protein standards were obtained from Bethesda Research Laboratories, Inc. (Bethesda, Md.). The gels were run at 60 mA constant current for 4 to 5.5 h. After electrophoresis the gels were prepared for Western blotting (30) with the Pharmacia Transphor system and according to the manufactur-

* Corresponding author.

TABLE 1. *Borrelia* and *Leptospira* species used

Isolate and strain or serovar	Source
B. burgdorferi	
ATCC 35210	*Ixodes dammini*
297	Human spinal fluid
P/Bi	Human spinal fluid
P/Gu	Human skin isolate
G25	*Ixodes ricinus*
Johnson tick	*Ixodes dammini*
B. burgdorferi-like strain 19865	Rabbit kidney
Borrelia sp. strain 11616	Shrew
B. coriaceae ATCC 43381	*Ornithodoros coriaceus*
L. interrogans serovars	
hardjo	
hardjoprajitno	Reference strain
harjo bovis	Bovine

ers' specifications. The nitrocellulose membranes were blocked and probed with antibody by standard procedures (6). Rabbit antiserum raised against *B. coriaceae* (titered at 1:12,000 by an enzyme-linked immunosorbent assay) was used to probe the blots at a dilution of 1:3,000. Biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, Calif.) and streptavidin-conjugated alkaline phosphatase (Kirkegaard and Perry, Gaithersburg, Md.) were then added according to manufacturers' specifications. The enzyme substrate, 5-bromo-4-chloro-3-indolyl phosphate, and Nitro Blue Tetrazolium (Kirkegaard and Perry) were added, and the reacting antigen-antibody complex was visualized.

RESULTS

B. coriaceae was characterized genetically and antigenically in this study. Figure 1 illustrates genomic DNA from *B. coriaceae* and several different *B. burgdorferi* isolates digested with *Hin*dIII and fractionated in a 0.7% agarose gel. Banding differences are present between all of the *Borrelia* isolates on this gel. The *B. coriaceae* isolate was distinct at virtually all molecular weight sizes from the genomes represented on this gel when digested with *Hin*dIII. Digestion with other endonucleases such as *Eco*RI, *Bam*HI, and *Hha*I further substantiated the overall differences seen in Fig. 1 (data not shown).

Figure 2A illustrates a further characterization of *B. coriaceae* and several other *B. burgdorferi* isolates digested with *Hin*dIII. The primary purpose of this gel was to transfer the DNA fragments to nylon membranes by the method of Southern (27) and to probe them with radiolabeled genomic *B. coriaceae* DNA. The *B. coriaceae* genomic DNA was underloaded (1 μg instead of 2.5 μg) to prevent overexposure of this lane when compared with the others. Figure 2B illustrates the autoradiograph of DNA transferred from the gel in Fig. 2A probed with genomically labeled *B. coriaceae*. Other than hybridization to self there is very little homologous hybridization to the other *B. burgdorferi* isolates. Figure 2C represents an overexposure of Fig. 2B, enhancing the visualization of the weakly hybridized bands of the heterologous genomes.

Figure 3 illustrates the extrachromosomal DNA present in *B. burgdorferi* and *B. coriaceae* by pulsed-field gel electrophoresis. Evidence of plasmids in several *Borrelia* species, including *B. coriaceae*, has been described previously (2, 3,

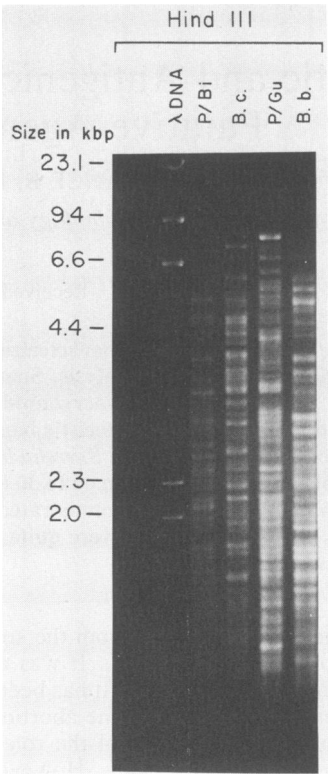

FIG. 1. *Hin*dIII restriction endonuclease digest of λ DNA, *B. burgdorferi* human spinal fluid isolate (P/Bi), *B. coriaceae* ATCC 43381, *B. burgdorferi* human skin isolate (P/Gu), and *B. burgdorferi* ATCC 35210.

10, 11). It appears from this gel that *B. coriaceae* contains several linear plasmids. This assumption is based on the fact that circular plasmids migrate very poorly in this system and that they migrate independent of the pulse field or switching time (5, 7, 18). Conversely, the migration of linear plasmids in this system is dependent upon these parameters. These bands remained in the same position relative to that of the linear lambda molecular weight markers under different switching conditions (data not shown). Thus, it is assumed that the multiple bands observed in the molecular size range of approximately 25 to 65 kilobase pairs are linear plasmids in *B. coriaceae*.

Antigens expressed by *B. coriaceae* were also characterized by Western blot analysis. Figure 4 illustrates antigens from *B. coriaceae*, *B. burgdorferi*, and *L. interrogans* serovar hardjo reacting to rabbit antiserum raised against whole cell *B. coriaceae*. Only a few weakly reacting bands could be seen of the *Leptospira* antigens. Both hardjo bovis and hardjoprajitno proteins reacted similarly with the antiserum. Antigens of 53, 44, 36, and 13 kilodaltons (kDa) were the most reactive for these organisms. Several strongly reacting antigens of the *B. burgdorferi* isolates were seen on the blot ranging from 48 to 30 kDa. As expected, the *B. coriaceae* antiserum reacted most strongly and most abundantly with homologous antigens. Several proteins were detected by the antiserum; those reacting the strongest were in the molecular size range of 37 to 33 kDa.

DISCUSSION

B. coriaceae has been reported to be the etiologic agent of epizootic bovine abortion (12, 16, 20, 21). However, it has

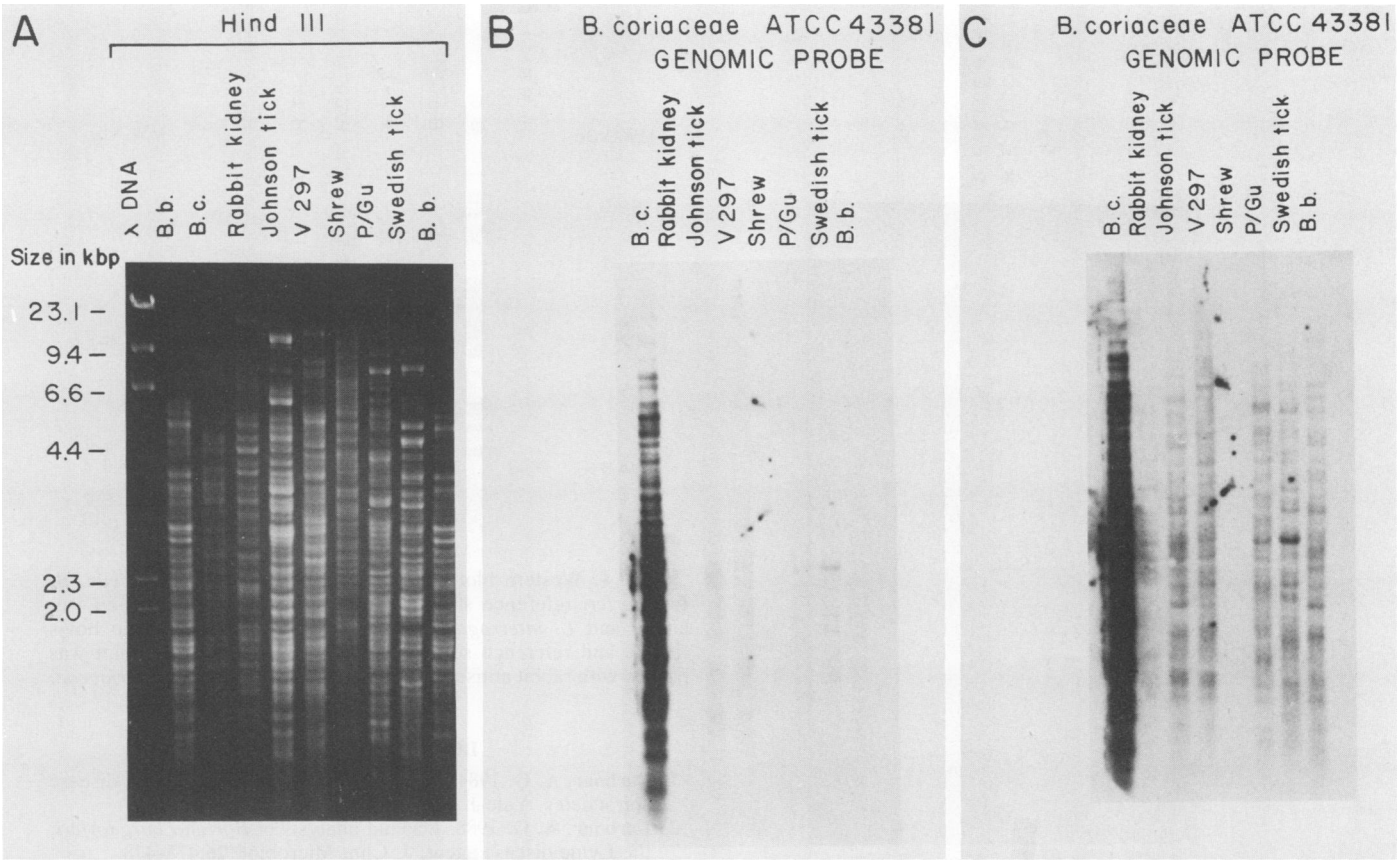

FIG. 2. (A) *Hin*dIII restriction endonuclease digest of λ DNA, *B. burgdorferi* ATCC 35210 (B.b.), *B. coriaceae* ATCC 43381 (B.c.), a *B. burgdorferi*-like isolate from a rabbit kidney, a *B. burgdorferi* isolate from *I. dammini* (Johnson tick), V297 (*B. burgdorferi* human spinal fluid isolate), an unclassified shrew isolate, a *B. burgdorferi* human skin isolate (P/Gu), a *B. burgdorferi* isolate from an *I. ricinus* tick from Sweden (G25), and *B. burgdorferi* ATCC 35210 (B.b.). (B) Autoradiograph of a southern blot of A minus the λ DNA and the first *B. burgdorferi* lane. The blot was probed with radiolabeled *B. coriaceae* reference strain DNA. (C) Overexposure of panel B, enhancing the lighter bands.

proven to be very difficult to isolate from the *Ornithodoros* tick and has yet to be cultured from an animal host. Difficulty of isolation and culture is also true for other genera of the *Spirochaetaceae* family, namely, *Treponema* spp., *Borrelia* spp., and *Leptospira* spp. Notwithstanding, studying the organism at the genetic and antigenic level is important to increasing our knowledge of it as a possible pathogen. Reported here for the first time is the characterization of *B. coriaceae* by restriction endonuclease analysis, Southern blots, DNA hybridization, pulsed-field gel electrophoresis, and Western blot analysis.

The restriction endonuclease analysis of the *B. coriaceae* genome appeared to be distinct when compared with those of genomic digests of other *Borrelia* isolates. The significance of the intraspecies differences between the *B. burgdorferi* isolates has been discussed elsewhere (R. B. LeFebvre, G. C. Perng, and R. C. Johnson, submitted for publication). Southern blot hybridization with radiolabeled *B. coriaceae* DNA demonstrated very little homology with other *B. burgdorferi* genomes. Based on these results and the low homology with other *Borrelia* species (11), it is conceivable that *B. coriaceae* genomic DNA could be used as a probe for the detection of the organism in potentially infected cows or aborted fetuses. Serological assays for the presence of the organism have been inconclusive in detecting an infection by *B. coriaceae*. Restriction endonuclease analysis could also prove to be useful in properly identifying spirochetes isolated from *Ornithodoros* ticks. This methodology has been shown to be specific in the identification of various other bacteria, including spirochetes (28, 29).

The results of the pulsed-field electrophoresis revealed the presence of several linear plasmids in *B. coriaceae*. Though it is rare to find linear extrachromosomal elements in eubacteria, it appears to be quite common in the genus *Borrelia*, having already been demonstrated in *B. hermsii* (23) and *B. burgdorferi* (2, 3). Linear plasmids have also been described in several *Streptomyces* strains (8, 13). Those characterized to date in *Borrelia* spp. contain genes encoding outer membrane antigens (3), whereas those in *Streptomyces* spp. encode antibiotic resistance (8). The function of linear plasmids in *B. coriaceae* is not yet known. However, it is of interest there are several of these plasmids when compared with the number in *B. hermsii* (23) or *B. burgdorferi* (2). Whether some of these extrachromosomal elements will be lost after subsequent passages in culture, as has been demonstrated with *B. burgdorferi* (2, 25), remains to be seen.

The antigen characterization of *B. coriaceae* was performed by immunoblot analysis. Except for a small number of antigens from *B. burgdorferi* and *L. interrogans* that were bound by the *B. coriaceae* antiserum, *B. coriaceae* appears to synthesize a unique and characteristic repertoire of antigens when compared with those synthesized by species of these other genera. However, *B. coriaceae* has only been isolated once, and therefore it remains to be demonstrated

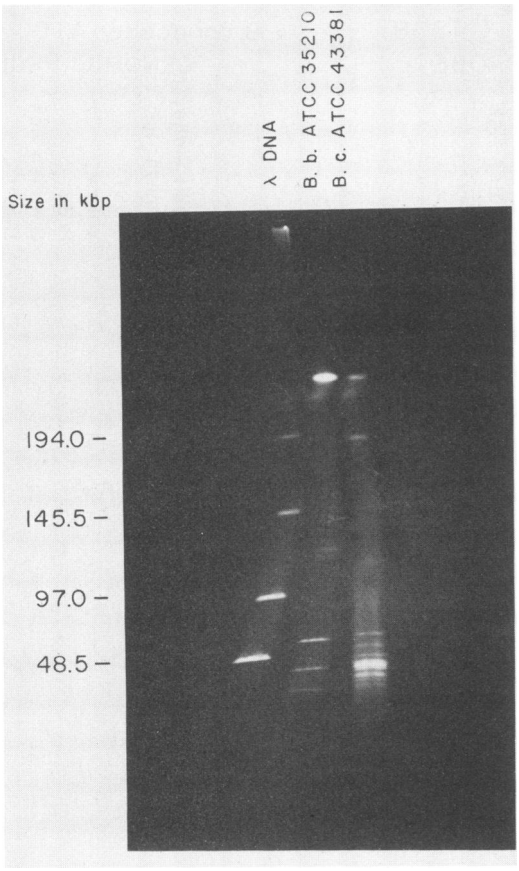

FIG. 3. Pulsed-field gel of λ DNA molecular weight concatemers, *B. burgdorferi* ATCC 35210, and *B. coriaceae* ATCC 43381.

that antigens expressed in future isolates will be similar to those identified here. The strongly reacting band of *B. burgdorferi* at 39 to 40 kDa may correspond to the flagellar antigen (4). It has been reported that a monoclonal antibody directed against the *B. burgdorferi* flagellar antigen (H9724) also reacts with an antigen from *B. coriaceae* (12). Due to this apparent similarity, it is probable that antibodies raised against whole-cell *B. coriaceae* would bind to the *B. burgdorferi* flagellar antigen. The *B. coriaceae* antiserum also bound a 29- to 30-kD antigen from all of the *B. burgdorferi* isolates. It is possible that this antigen represents an outer surface protein (designated OspA) (9). This would corroborate the findings of Lane and Manweller in their demonstration that a monoclonal antibody (H5332) reacted against the OspA antigen of *B. burgdorferi* bound spirochetes from tissue smears of *O. coriaceus* ticks (15).

It appears that the organism is highly immunogenic, in light of the number of proteins reacting to the antiserum. Whether any of these antigens elicits a protective immune response from the host has not been determined. *B. coriaceae*, like other pathogenic spirochetes, may be able to evade the host defense mechanisms despite a strong humoral response.

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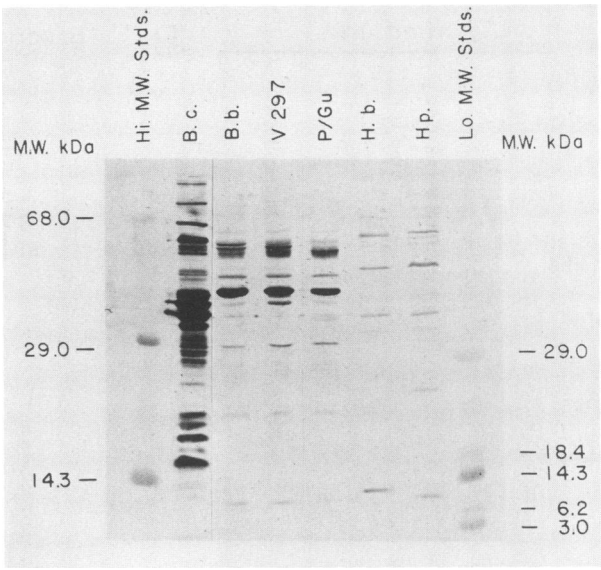

FIG. 4. Western blot of whole cell lysates of *B. coriaceae*, *B. burgdorferi* reference strain, V297 and P/Gu (*B. burgdorferi* isolates), and *L. interrogans* serovar hardjo genotype hardjo bovis (H.b.), and reference serovar hardjoprajitno (Hp.). The blot was probed with rabbit antiserum raised against whole-cell *B. coriaceae*.

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