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Antigenically Variable *Borrelia burgdorferi* Isolated from Cottontail Rabbits and *Ixodes dentatus* in Rural and Urban Areas

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Spirochetes were isolated from 71 subadult *Ixodes dentatus* removed from cottontail rabbits captured in Millbrook, N.Y., and in New York, N.Y. Spirochetes were also cultured from kidney tissues of six rabbits. While all isolates reacted with monoclonal antibody H9724, which identifies the spirochetes as borreliae, more than half did not bind with antibody H5332 and even fewer reacted with H3TS, both of which were produced to outer surface protein A of *Borrelia burgdorferi*. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein profiles of three isolates differed from one another and from all previously characterized *B. burgdorferi* strains from humans, ticks, and wildlife in North America. The 12 periplasmic flagella that originated subterminally from each pointed end of a rabbit *Borellia* isolate contrasted with the 11 or fewer flagella for *B. burgdorferi* reported previously from North America. Although DNA homology and restriction endonuclease analysis also revealed differences among a rabbit kidney isolate, an *I. dentatus* isolate, and *B. burgdorferi* B31, similarities were sufficient to lead us to conclude that the borreliae in rabbits and *I. dentatus* are *B. burgdorferi*. Enzyme-linked immunosorbent assay titers of sera from humans with diagnosed Lyme disease to rabbit tick *B. burgdorferi* were often similar to one another and to those recorded for a reference *B. burgdorferi* strain.

Borrelia burgdorferi (27), the causative agent for Lyme disease (19), is the only tick-borne spirochete thus far isolated in the eastern United States. The isolates from humans (14, 47), *Ixodes dammini* (2, 7, 19, 28, 47), *I. pacificus* (15, 20), rodents, (2, 3, 6, 7, 16, 21, 23, 34, 35), and a raccoon, *Procyon lotor* (7), had outer surface protein A (OspA), OspB, and flagellin proteins with approximately similar molecular weights (10–13). One *B. burgdorferi* isolate from *I. pacificus* had an abundant protein with a molecular weight of about 25,000, but lacked OspA and OspB proteins (15), and the strain from a bird (4) had a slightly lower-molecular-weight OspA protein and apparently lacked an OspB protein (11). We now report the culturing of antigenically variable borreliae from *I. dentatus* and its primary mammalian host, the cottontail rabbit (*Sylvilagus floridanus*), from rural and urban areas in the northeastern United States.

MATERIALS AND METHODS

Rabbit and tick collections. Cottontail rabbits were captured in wooden box traps in the New York Botanical Garden in New York, N.Y., and from the Mary Flagler Cary Arboretum, Millbrook, N.Y., from August to October 1985. Live, captured rabbits were transferred to wire cages at the arboretum and placed over water. Engorged ticks, which dropped off each rabbit, were removed from the water daily and later identified to stage and species. Some engorged larval *I. dentatus* molted to nymphs in the laboratory.

***Borrelia* isolation procedures.** Attempts were made to isolate borreliae from *I. dentatus* (engorged larvae, $n = 54$; engorged nymphs, $n = 74$; engorged females, $n = 3$; unengorged nymphs, $n = 39$), *Haemaphysalis leporispalustris* (engorged larvae, $n = 20$; engorged nymphs, $n = 52$; engorged females, $n = 31$; males, $n = 45$), and blood and tissues of liver, spleen, or kidneys of 19 cottontail rabbits. Midgut tissues were dissected in a drop of Barbour-Stonner-Kelly (8) medium after each tick had been surface cleansed for about 1 min in 70% isopropyl alcohol. Tissues were subsequently transferred to duplicate tubes of Barbour-Stonner-Kelly medium containing 0.1% agarose (SeaKem LE; FMC Corp., Marine Colloids Div., Rockland, Maine), 1-cysteine hydrochloride (0.023%), DL-dithiothreitol (0.015%), and superoxide dismutase (0.002%) (26). The antibiotics rifampin (50 mg/ml) or 5-fluorouracil (230 mg/ml) and kanamycin sulfate (8 mg/ml) were added to one of each pair of culture tubes (28). Blood and tissues were aseptically removed from rabbits and inoculated into duplicate tubes of Barbour-Stonner-Kelly medium with or without the above-named antibiotics by procedures described for rodents (3, 26). Inoculated media were kept at 31°C for 3 to 6 weeks and examined for borreliae by dark-field microscopy.

Serotyping isolates by IFA staining. Indirect fluorescent-antibody (IFA) reactions of 59 isolates from *I. dentatus* and 3 isolates from cottontail rabbits to three murine monoclonal antibodies were recorded. The antibodies tested were H5332 (13) and H3TS (11), which react with different epitopes of the approximately 31,000-molecular-weight OspA protein of *B. burgdorferi*. The third antibody, H9724, responds to flagellin antigens that are common to all species of *Borrelia* (10) thus far tested. Flagellin has an approximate molecular weight in *B. burgdorferi* of 41,000.

SDS-PAGE analysis. One *I. dentatus* and two rabbit isolates were characterized by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and compared with a reference *B. burgdorferi* isolate (strain CT2591) from a white-footed mouse, *Peromyscus leucopus* (7). After being washed twice in phosphate-buffered saline solution with 5 mM $MgCl_2$ and having protein contents determined, spiro-

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chetes were boiled for 5 min in SDS incubation buffer and tested by SDS-PAGE in a Hoefer SE600 vertical gel unit (Hoefer Scientific Instruments, San Francisco, Calif.). Gels were stained with Coomassie brilliant blue R-250, and the major proteins were compared with molecular weight standards (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Western blot (immunoblot) analysis. Proteins from the CT2591 strain of *B. burgdorferi*, one *I. dentatus* isolate, and two rabbit isolates were transferred from an SDS-PAGE gel to a nitrocellulose membrane (Transphor transfer medium; 4.5-μm pores; Hoefer Scientific Instruments.) (48). A Hoefer Transphor (TE52) cell containing 192 mM glycine, 26 mM Tris base, and 20% methanol in distilled water and fitted with a Hoefer power lid was used to transfer the proteins in 45 min at 0.6 A. After drying, the membrane was saturated with blocking solution (2% bovine serum albumin [fraction V], 0.25% gelatin, 5 mM EDTA disodium salt, 0.05% Nonidet P-40 in 20 mM Tris–500 mM NaCl [Tris-buffered saline]) and then incubated for 2 h in a 1:100 dilution of murine sera containing monoclonal antibody H5332, H3TS, H6831 (12), or H9724 in blocking solution. After two 15-min washes in blocking solution, the proteins on the membrane were exposed to horseradish peroxidase-conjugated goat anti mouse immunoglobulin G (Tago, Inc., Burlingame, Calif.) diluted 1:500 in Tris-buffered saline for 2 h. Following two washings in blocking solution, proteins on the membrane that bound with the antibody were stained with horseradish peroxidase color development reagent (enzyme immunoassay purity reagent; Bio-Rad Laboratories, Rockville Centre, N.Y.).

Isolation of genomic DNA. Whole-cell DNA was isolated from the type strain of *B. burgdorferi* B31, ATCC 35210; a rabbit kidney isolate, 19865, and an *I. dentatus* isolate, 19941, by the method of LeFebvre et al. (33).

DNA homology studies. Whole-cell genomic DNAs from rabbit isolate 19865 and *I. dentatus* isolate 19941 were digested with *Hin*dIII and labeled with radioactive [α-^{32}P]-dCTP by nick translation (38). The labeled DNAs from the rabbit and *I. dentatus* isolates were used to quantify the DNA homology shared between the genomes of these spirochetes, using the method of Hamamoto et al. (24). Briefly, the unlabeled DNA in 2× sodium chloride-sodium citrate (diluted from a 20× stock of 3 M sodium chloride plus 0.3 M sodium citrate; 1× sodium chloride-sodium citrate is 0.15 M NaCl plus 0.015 M sodium citrate) was denatured by heating in a boiling-water bath for 5 min, trapped on a nitrocellulose filter by suction, and fixed by heating at 85°C in a vacuum oven. Two different parameters were used to determine the degree of homology shared between these organisms. The rabbit kidney isolate was assayed at 50 and 65°C. Due to the low G+C content of organisms in the *Borrelia* genus (approximately 28%), the *I. dentatus* isolate was assayed for homology to the other two *Borrelia* isolates at 60 and 75°C (41). The hybridizations were carried out for 60 h at the respective temperatures. The filters were then washed in several washes of 2× sodium chloride-sodium citrate. A 10-ml amount of scintillation fluor was added, and the radioactivity was assayed in a scintillation counter.

Restriction endonuclease analysis of genomic DNA. Whole-cell DNA from the three organisms was digested with restriction endonucleases *Hin*dIII, *Eco*RI, *Bam*HI, and *Hha*I following the manufacturer's specifications (Bethesda Research Laboratories). The resulting fragments were fractionated by electrophoresis in a 0.7% agarose gel at 60 V for 15.5 h. The DNA was visualized and photographed after staining with ethidium bromide and illumination by UV radiation.

Southern blot analysis. Following photography, the DNA in the gel was transferred to a nylon membrane by the method of Southern (44). The labeled DNA described above from the rabbit tick isolate (*I. dentatus* 19941) was used to probe the Southern blot to identify homologous fragments of the genomes of these organisms.

ELISA of human sera. Titers of persons diagnosed with Lyme disease were determined by testing sera against *B. burgdorferi* CT2591, two isolates from cottontail rabbits, and two isolates from *I. dentatus*. Blood samples were obtained from 30 persons with erythema migrans, from 20 patients with cardiac, neurological, or arthritic disorders compatible with Lyme disease but who had no history of skin lesions, and from 17 persons not known to have a history of Lyme disease or other spirochetal infections.

Procedures for preparation of whole-cell antigen and the enzyme-linked immunosorbent assay (ELISA) have been described previously (37). Titers were determined after applying a goat anti-human immunoglobulin G antibody conjugated with horseradish peroxidase (Kirkegaard and Perry, Gaithersburg, Maryland) and diluted to 1:800 with phosphate-buffered saline solution containing 0.01% aqueous thimerosal. All antigen preparations were adjusted to contain an equivalent quantity of protein (ca. 80 μg/ml) after analysis with a commercially available assay (Bio-Rad). Optical density values were measured by a microplate reader with net absorbance values of >0.21, >0.13, and ≥0.08 being judged as positive for the corresponding serum dilutions of 1:160, 1:320, and ≥1:640.

Electron microscopy. Suspensions of washed but unfixed whole-cell borreliae of rabbit isolate 19865 were placed on Formvar-coated grids, stained with 2% aqueous phosphotungstic acid (pH 7.0), and examined with a Zeiss EM-10 electron microscope at an accelerating voltage of 80 kV. Suspensions of *I. dentatus* isolate 21040 were also fixed, embedded, and examined in thin section by procedures described previously (5).

RESULTS

Isolations of borreliae. Spirochetes were isolated from 71 subadult *I. dentatus* collected at both study sites, including 26 unfed nymphs which had been collected as engorged larvae and allowed to molt to nymphs in the laboratory (Table 1). Spirochetes were not recovered from immature or

TABLE 1. *Borrelia* isolation results from *I. dentatus* that had fed on cottontail rabbits at the Mary Flagler Cary Arboretum, Millbrook, N.Y., and the New York Botanical Garden, New York, N.Y., 1985

Collection site	Tick growth stage	Total no. of ticks tested	No. (%) of tick cultures		
			Positive	Negative	Contaminated
Millbrook	Engorged larva	53	19 (40)	25 (50)	9 (10)
	Engorged nymph	73	25 (34)	20 (27)	28 (39)
	Unfed nymph	39	26 (66)	5 (13)	8 (21)
	Engorged female	1	0 (0)	0 (0)	1 (100)
New York City	Engorged larva	1	1 (100)	0 (0)	0 (0)
	Engorged nymph	1	0 (0)	0 (0)	1 (100)
Total		168	71 (42)	50 (30)	47 (28)

TABLE 2. *Borrelia* isolation results from tissues of cottontail rabbits captured at the New York Botanical Garden, New York, N.Y., and the Mary Flagler Cary Arboretum, Millbrook, N.Y., 1985

Collection site	No. of positive rabbits/ no. examined	No. of isolations/no. of tissues from different hosts				
		Blood	Spleen	Left kidney	Right kidney	Liver
New York City	1/3	ND[a]	0/3	1/3	0/3	0/1
Millbrook	5/16	0/4	0/4	3/13	3/12	0/8

[a] ND, Not done.

adult *H. leporispalustris* collected from rabbits captured in the Mary Flagler Cary Arboretum (4 ticks) and the New York Botantical Garden (82 ticks). Tissues from an additional 55 *H. leporispalustris* produced contaminated cultures.

Borreliae also were isolated from the kidneys of six cottontail rabbits captured at the two sites (Table 2).

Serotyping isolates by IFA tests. Three variants, based upon IFA reactions to three monoclonal antibodies, were identified from cottontail rabbits and *I. dentatus* (Table 3). Over half of the isolates reacted only with H9724, while the remaining 27 isolates bound also with antiserum to OspA H5332. Sixteen of the tick isolates reacted with all three monoclonal antibody preparations.

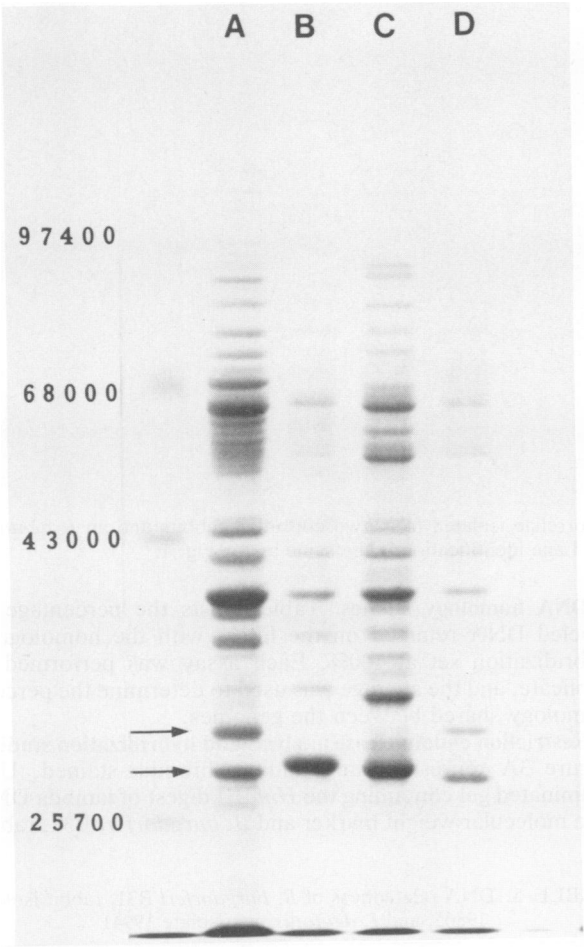

FIG. 1. Coomassie brilliant blue-stained proteins of whole-cell lysates of *B. burgdorferi* and of borreliae isolated from two cottontail rabbits and one *I. dentatus* nymph. Lane A, *B. burgdorferi* CT 2591 from a white-footed mouse; lane B, borreliae (strain 19857) from a cottontail rabbit; lane C, borreliae (strain 19865) from a cottontail rabbit; lane D, borreliae from an *I. dentatus* nymph (strain 19941). Molecular weights are shown on the left. Arrows identify the OspA and OspB proteins of *B. burgdorferi*.

TABLE 3. IFA reactions of borreliae isolated from *I. dentatus* and cottontail rabbits with monoclonal antibodies

No. of isolates from:		Reactions with monoclonal antibodies		
I. dentatus	Rabbit	OspA		Flagellin (H9724)
		H5332	H3TS	
32	2	−	−	+
10	1	+	−	+
16		+	+	+

The antigenic composition of borreliae cultured from two cottontail rabbits was compared with that of the borreliae isolated from *I. dentatus* that had naturally fed on the rabbits. The reactivity to these monoclonal antibodies to cells from five larval and one nymphal *I. dentatus* from rabbit 6 differed from that of the borreliae isolated from their host rabbit (Table 4). While the rabbit isolate reacted with two preparations of monoclonal antibodies, the tick isolates bound to one or all three of the monoclonal antibodies tested. The cultures from rabbit 8 and from one nymphal and five larval ticks that had fed on this rabbit all reacted with antibody H9724 only. Two other cultures from larval ticks from this rabbit bound with two and with all three of the antibodies, respectively. The standard *B. burgdorferi* isolate (CT2591) reacted with antibodies H5322, H3TS, and H9724.

SDS-PAGE analysis. Three of the isolates (two rabbit, 19857 and 19865, and one *I. dentatus* 19941) were compared with *B. burgdorferi* CT2591 by SDS-PAGE. These three isolates differed from one another and from *B. burgdorferi* (10% acrylamide gel in Fig. 1), although all isolates had

TABLE 4. IFA reactions of borreliae isolated from engorged ticks that had fed on two naturally infected cottontail rabbits and of *B. burgdorferi* isolated from blood of a white-footed mouse

Rabbit no.	Culture		IFA reactions to monoclonal antibodies		
	Sources	No.	OspA		Flagellin (H9724)
			H5332	H3TS	
6	Rabbit kidney	19857	+	−	+
6	*I. dentatus* larva	21123	−	−	+
6	*I. dentatus* larva	21126	−	−	+
6	*I. dentatus* larva	21133	−	−	+
6	*I. dentatus* larva	21136	−	−	+
6	*I. dentatus* larva	21038	+	+	+
6	*I. dentatus* nymph	21040	+	+	+
8	Rabbit kidney	19865	−	−	+
8	*I. dentatus* nymph	19941	−	−	+
8	*I. dentatus* larva	19950	−	−	+
8	*I. dentatus* larva	19952	−	−	+
8	*I. dentatus* larva	19954	−	−	+
8	*I. dentatus* larva	19955	+	+	+
8	*I. dentatus* larva	19957	+	−	+
8	*I. dentatus* larva	19959	−	−	+
8	*I. dentatus* larva	21122	−	−	+
	B. burgdorferi	CT2591	+	+	+

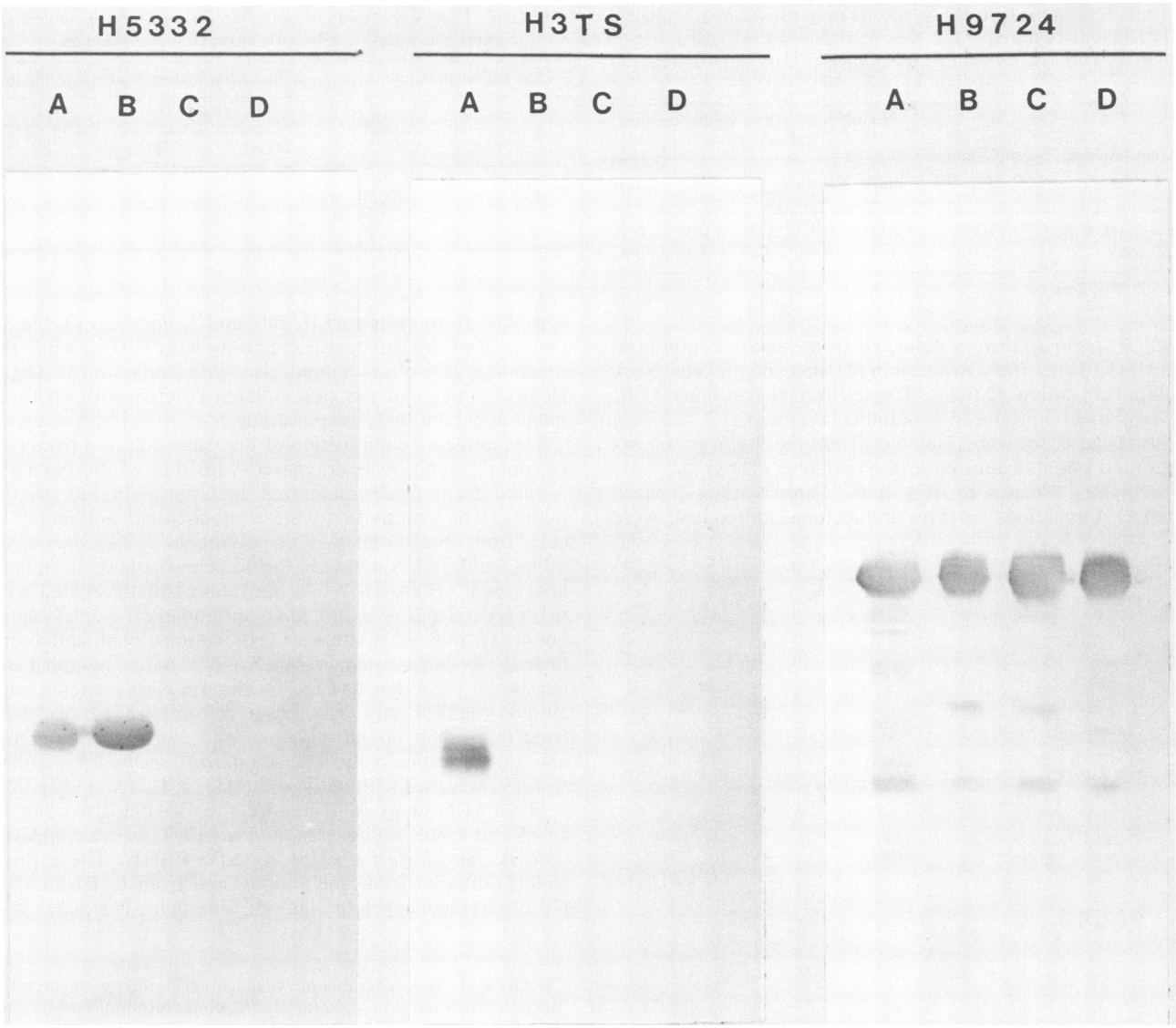

FIG. 2. Western blots of whole-cell lysates of *B. burgdorferi* and of borreliae isolated from two cottontail rabbits and an *I. dentatus* nymph. The blots were reacted with antibodies H5332, H3TS, and H9724. Lane identification is the same as for Fig. 1.

protein bands of 60,000 and 41,000. Two other major protein bands of *B. burgdorferi* have approximate molecular weights of 34,000 (OspB) and 31,000 (OspA) (identified by arrows in Fig. 1). Isolate 19857 from rabbit 6 had an OspA with a molecular weight of approximately 31,250. Kidney isolate 19865 from rabbit 8 possessed major protein bands with molecular weights of about 35,500 and 31,000. The *I. dentatus* 19941 isolate had polypeptides with molecular weights of about 34,250 and 30,500.

Western blot analysis. Western blots were used to show reactivity of polypeptides of the *B. burgdorferi*, two rabbit, and one *I. dentatus* borreliae that bound with monoclonal antibodies by IFA methods. The approximately 41,000-molecular-weight flagellin protein of all four isolates reacted with the antiserum (H9724) directed to these polypeptides (Fig. 2). The OspA protein that reacts with H5332 was identified only in rabbit kidney isolate 19857 and *B. burgdorferi* CT2591, but the polypeptide which bound to antibody H3TS was evident only in *B. burgdorferi* CT2591. None of the isolates reacted with antibody H6831 directed to the OspB protein of *B. burgdorferi* B31.

DNA homology studies. Table 5 lists the percentage of labeled DNA retained on the filters with the homologous hybridization set at 100%. Each assay was performed in triplicate, and the average was used to determine the percent homology shared between the genomes.

Restriction endonuclease analysis and hybridization studies. Figure 3A represents an ethidium bromide-stained, UV-illuminated gel containing the *Hin*dIII digest of lambda DNA as a molecular weight marker and *B. burgdorferi* B31, rabbit

TABLE 5. DNA relatedness of *B. burgdorferi* B31, rabbit isolate 19865, and *I. dentatus* tick isolate 19941

Unlabeled DNA	% Relative binding of labeled DNA from:			
	Rabbit isolate		Tick isolate	
	50°C	65°C	60°C	75°C
B. burgdorferi	91	61	64	57
Tick isolate	100	80	100	100
Rabbit isolate	100	100	79	82

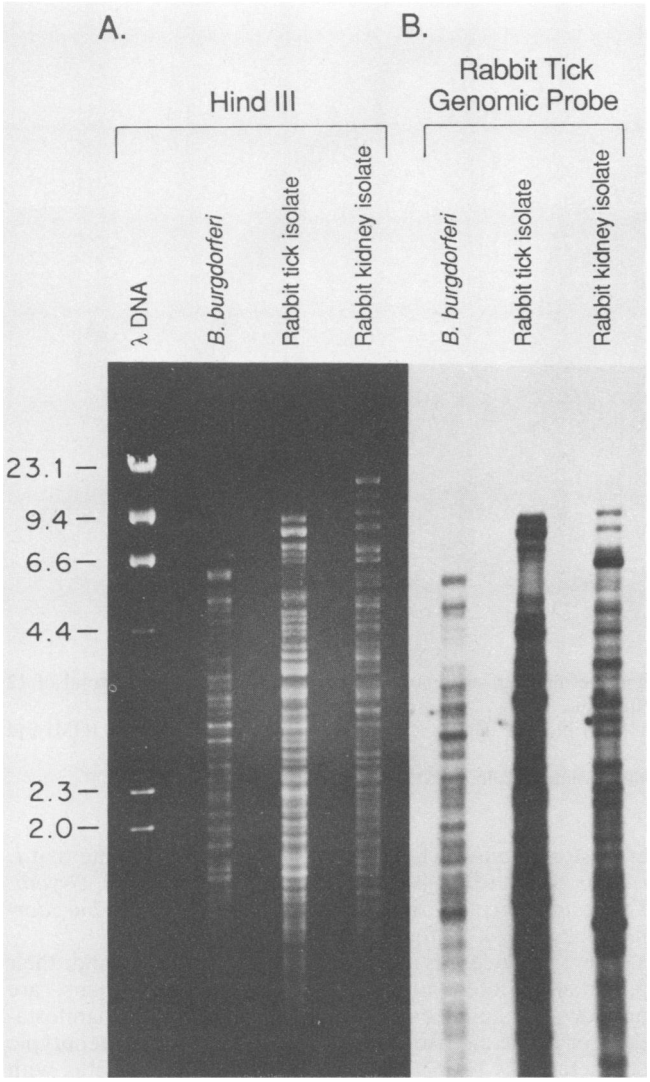

FIG. 3. Restriction endonuclease analysis and hybridization study. (A) Ethidium bromide-stained 0.7% agarose gel of *Hin*dIII-digested whole-cell DNA from *B. burgdorferi*, rabbit tick isolate (*I. dentatus* 19941), and rabbit kidney isolate (strain 19865). (B) Southern blot of the DNA in panel A probed with radiolabeled whole-cell DNA from the rabbit tick isolate.

tick (=*I. dentatus* 19941) isolate, and rabbit kidney (19865) isolate genomic DNAs. Clearly, there is considerable disparity between these organisms regarding their fragment distribution after digestion with this endonuclease. However, the Southern blot and hybridization of this gel with labeled tick isolate genomic DNA show a high degree of homology among the bands (Fig. 3B).

Figure 4 illustrates the restriction endonuclease analysis of *B. burgdorferi*, rabbit tick, and rabbit kidney isolates digested with *Hha*I, *Eco*RI, and *Bam*HI, respectively. The individual patterns of the restricted fragments for the three genomes are different, although there appears to be a closer similarity in the overall patterns than with the *Hin*dIII digest. For example, the *Bam*HI digest shows a large number of common bands among the three genomes.

Electron microscopy. The ultrastructure of the rabbit borreliae was determined by transmission electron microscopy. As many as 12 periplasmic flagella originated subterminally from each pointed end (Fig. 5). The insertion points were more or less in parallel with the long axis, and all flagella were enclosed within an outer membrane (Fig. 6). Spirochete cells measured 0.19 to 0.28 μm in diameter. Negatively stained bacteria were 10.6 to 11.4 μm in length with about five spirals (Fig. 7). Wavelength was 2.1 to 2.7 μm.

ELISA. The reactivity of antibodies from patients diagnosed with Lyme disease to *B. burgdorferi* and borreliae from rabbits and *I. dentatus* was assessed. Representative titers of sera from persons diagnosed with Lyme disease to five antigens are shown in Table 6. Titers varied usually by twofold among *B. burgdorferi* CT2591, two rabbit isolates (19857 and 19865), and two *I. dentatus* isolates (21039 and 19941). Sera from 17 patients with no known history of Lyme disease were negative to all five antigens tested.

DISCUSSION

The spirochetes cultured from cottontail rabbits and their ticks, *I. dentatus*, are, to our knowledge, the first borreliae to be recovered from these species. All isolates reacted with antiflagellar antibody H9724, verifying that they belong to the genus *Borrelia* (10). The DNA homology studies confirmed that these organisms are closely related and should be identified as *B. burgdorferi*, i.e., rabbit tick *B. burgdorferi*. This conclusion is supported by the fact that species are defined as groups of organisms which share a relatively high

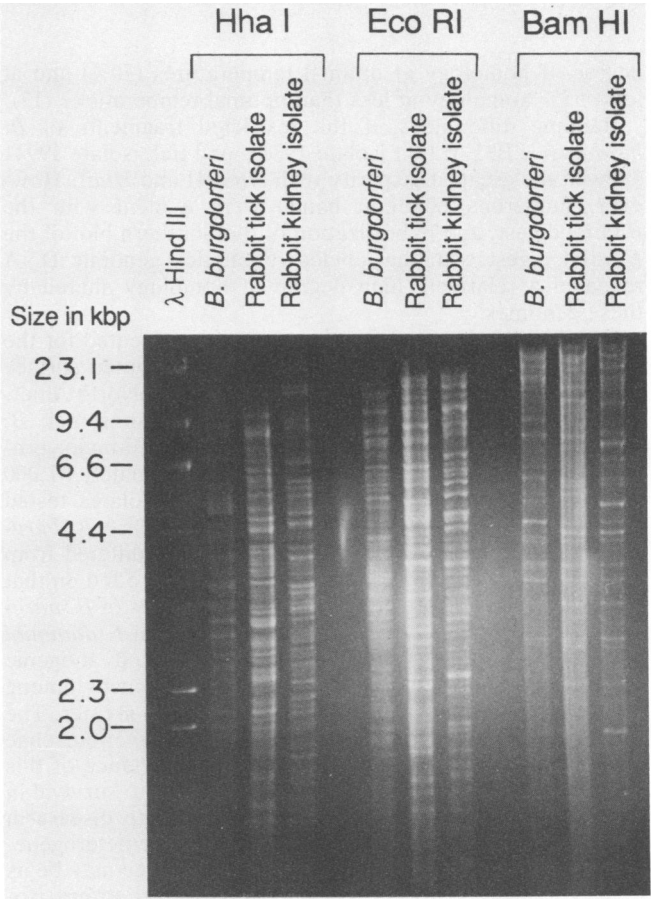

FIG. 4. Ethidium bromide-stained gel of *B. burgdorferi*, rabbit tick isolate (*I. dentatus* 19941), and rabbit kidney isolate (strain 19865) digested with *Hha*I, *Eco*RI, and *Bam*HI. kbp, Kilobase pairs.

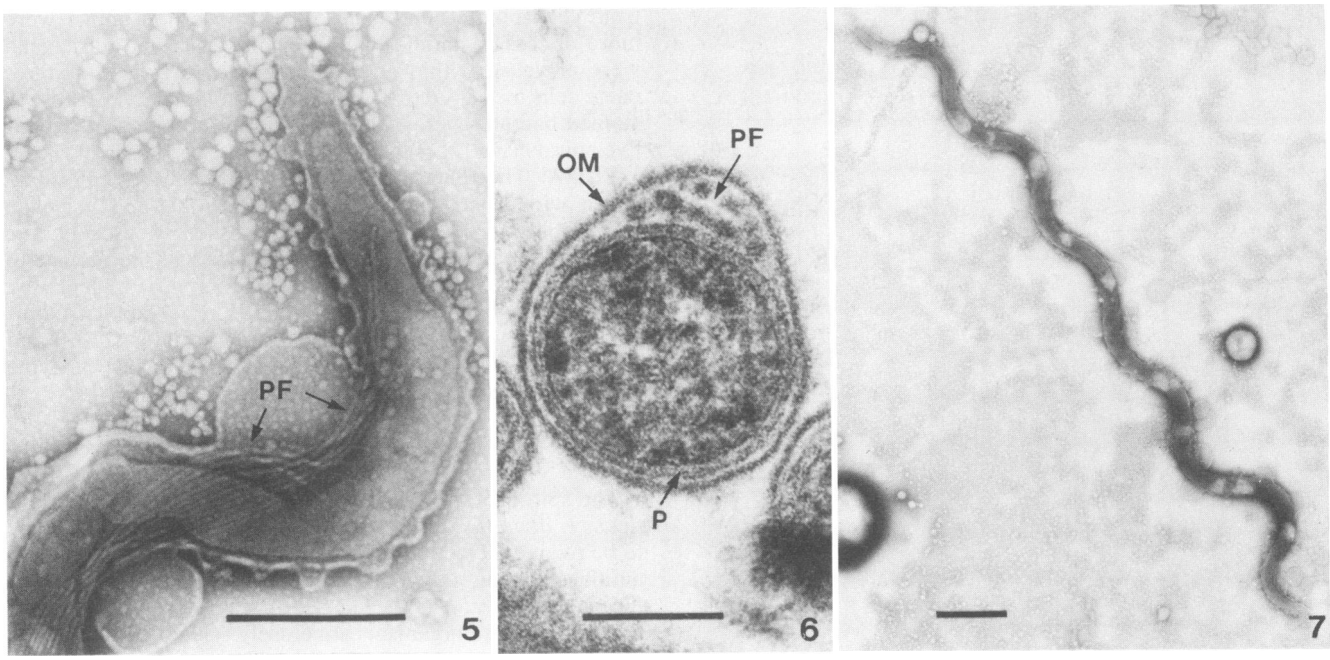

FIG. 5. Pointed end of a rabbit kidney isolate (strain 19865) showing subterminal insertions of periplasmic flagella (PF). A total of 12 periplasmic flagella are visualized. Unfixed and negatively stained. Bar, 0.5 μm.

FIG. 6. Cross section of *I. dentatus* isolate (strain 21040) showing several periplasmic flagella (PF) between the outer membrane (OM) and peptidoglycan layer (P). Bar, 0.1 μm.

FIG. 7. Unfixed, negatively stained micrograph of a whole rabbit kidney isolate (strain 19865). Bar, 1.0 μm.

degree of homology at optimal temperatures (70%) and at least 55% homology at less than optimal temperatures (17).

Banding differences of the restricted fragments of *B. burgdorferi* B31, rabbit isolate 19865, and tick isolate 19941 showed the greatest disparity with *Hin*dIII and *Hha*I. However, numerous common bands were evident with the *Bam*Hi digest, and hybridization of the Southern blot of the *Hin*dIII digest with the labeled rabbit tick genomic DNA revealed a relatively high degree of homology shared by these genomes.

Considerable antigenic variation was documented for the rabbit tick *B. burgdorferi*, which differ in outer surface protein composition from previously described North American isolates (11, 15). None of the three rabbit tick *B. burgdorferi* examined by SDS-PAGE possessed major protein bands with molecular weights of approximately 31,000 and 34,000. Protein compositions of three isolates tested differed from one another and from our reference *B. burgdorferi*. Even a culture from a rabbit kidney differed from cultures derived from larval *I. dentatus* that had fed on that particular rabbit. Antigenically different isolates of *B. burgdorferi* had previously been reported for larval *I. dammini* and its host, the white-footed mouse (2), and antigenic changes occur in culture (42), but the differences among isolates from rabbits and their ticks were much greater. The significance of antigenic variation of relapsing fever borreliae has recently been reviewed (9), but the importance of this phenomenon among rabbit tick *B. burgdorferi* to survival in their vertebrate and tick reservoir hosts and to disease in humans and domestic animals is unknown. The heterogeneity among major protein bands in these borreliae may be as diverse as has been reported in *B. burgdorferi* strains isolated from *I. ricinus* and humans in Europe (11, 46, 49, 50), many of which also do not react with monoclonal antibody H3TS (11). Animal reservoirs of *B. burgdorferi* in Europe are not well known (1, 31), but it is interesting to note that *I. ricinus* feeds extensively on the European rabbit *Oryctolagus cuniculus* (39). Possibly, variation among *B. burgdorferi* isolates will be found in these rabbits.

The virulence of rabbit tick *B. burgdorferi* and their potential to infect other animals, including humans, are unknown. It has been suggested that particular manifestations of Lyme disease may be related to specific phenotypic characteristics (11, 25, 46, 49, 50). While spirochetes with major proteins similar in molecular weight to those shown for borreliae from rabbits and *I. dentatus* have not been isolated from humans in the United States (14, 47), ELISA titers of sera from humans with diagnosed Lyme disease to these borreliae were often, though not always, similar to one another and to those recorded when our reference *B. burgdorferi* was tested. Clearly, immunoglobulins present in humans with diagnosed Lyme disease react with the rabbit tick *B. burgdorferi* isolates. Inasmuch as species of *Borrelia* have similar antigens such as those in the periplasmic flagella (10), serologic cross-reactivity among variants of borreliae is to be expected.

Competent tick vectors that could transmit rabbit tick *B. burgdorferi* to humans or domestic animals are unknown. *I. dentatus*, which transmits these borreliae transstadially, feeds almost exclusively on rabbits and birds (29) and rarely on humans (43). However, the important tick vectors of *B. burgdorferi* in North America (i.e., *I. dammini* [19], *I. pacificus* [20], and *I. scapularis* [36]) also feed on rabbits (22, 30, 45) and possibly could transfer these borreliae to humans. Furthermore, jackrabbits (*Lepus* spp.) may also be a reservoir for *B. burgdorferi* (32, 40) and a source of infection for additional tick species (32).

The etiologic agents of tick-borne borrelioses are considered to be contracted primarily in rural areas (18). Our isolation of borreliae from cottontail rabbits and their ticks

TABLE 6. ELISA titers (immunoglobulin G) of sera from 23 different patients diagnosed with Lyme disease[a] to *B. burgdorferi* CT2591 and rabbit tick *B. burgdorferi* isolates from *I. dentatus* and from kidneys of cottontail rabbits

Patient	Titers of antibody to isolates from:				
	White-footed mouse (*B. burgdorferi* CT2591)	*I. dentatus*		Cottontail rabbit	
		Larva (21039)[b]	Nymph (19941)	Kidney (19857)	Kidney (19865)
1	2,560	2,560	1,280	2,560	2,560
2	5,120	10,240	10,240	10,240	10,240
3	10,240	5,120	10,240	5,120	10,240
4	640	N[c]	N	N	640
5	10,240	5,120	5,120	5,120	10,240
6	5,120	2,560	2,560	2,560	5,120
7	20,480	20,480	20,480	20,480	40,960
8	10,240	10,240	10,240	20,480	40,960
9	10,240	5,120	10,240	5,120	5,120
10	5,120	5,120	5,120	10,240	10,240
11	640	N	N	N	640
12	10,240	5,120	5,120	5,120	10,240
13	640	N	N	N	2,560
14	640	640	640	640	1,280
15	2,560	1,280	2,560	2,560	2,560
16	1,280	640	640	640	1,280
17	2,560	640	640	640	640
18	5,120	2,560	1,280	2,560	5,120
19	5,120	2,560	2,560	2,560	2,560
20	2,560	2,560	1,280	2,560	2,560
21	5,120	2,560	2,560	2,560	2,560
22	1,280	1,280	1,280	1,280	2,560
23	5,120	10,240	5,120	5,120	5,120

[a] Patients 1 to 14 developed erythema migrans; patients 15 to 23 had no clinical history of erythema migrans, but they did present with arthritic or nervous system disorders compatible with symptoms of Lyme disease.
[b] Isolate number is given in parentheses.
[c] N, Negative.

collected in New York, N.Y., suggests the possibility that borreliae could be contracted even in urban areas if suitable vectors and reservoir hosts exist. Thus, physicians should be aware that persons presenting with symptoms of borreliosis but without a travel history into rural, tick-infested regions could, on occasion, have acquired borreliosis from a tick bite wholly within an urban setting.

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