JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1993, p. 2038–2043
0095-1137/93/082038-06$02.00/0

Vol. 31, No. 8

Isolation and Characterization of *Borrelia burgdorferi* from Blood of a Bird Captured in the Saint Croix River Valley

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Received 23 December 1992/Accepted 4 May 1993

Field investigations were conducted to further evaluate the role of birds in the maintenance and dissemination of *Borrelia burgdorferi*. Blood specimens were taken from 39 passerine birds of 17 species captured during June 1991 at the Saint Croix National Riverway in Wisconsin, and one isolate, WI91-23, was cultured from an adult song sparrow (*Melospiza melodia*). This isolate was shown to be infectious for *Peromyscus leucopus* and *Mesocricetus auratus* (golden hamster). Isolate WI91-23 was confirmed as *B. burgdorferi* by immunofluorescence assay by using species-specific anti-OspA monoclonal antibodies H3TS and H5332 and anti-OspB antibody H5TS. Isolate WI91-23 was compared with *Borrelia anserina* Es, *Borrelia hermsii* MAN-1, and other *B. burgdorferi* strains (ATCC 53210, CT-1, and *Catharus fuscescens* [veery] liver 10293). Pulsed-field gel electrophoresis of in situ-lysed spirochetes revealed that the DNA plasmid profile of WI91-23 was most similar to those of plasmids from *B. burgdorferi* and most different from those of plasmids from *B. anserina* and *B. hermsii*. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis indicated that the protein profile of WI91-23 was like that of other *B. burgdorferi* strains studied, with dominant proteins corresponding to OspA and OspB, and that it differed from the protein profiles of *B. anserina* and *B. hermsii*. These findings indicate that passerine birds may serve as reservoirs for *B. burgdorferi*.

Lyme disease, which is caused by the spirochete *Borrelia burgdorferi*, continues to be the major arthropod-associated disease in the United States (31), despite numerous efforts to prevent and control it. Three major foci are now recognized in the United States; the Northeast, the upper Midwest, and the Pacific coastal states (31). The disease was first recognized in the Midwest, in northwestern Wisconsin in 1970 (34); this was followed by the reporting of a cluster of cases there in 1978 (14). During the next decade, Lyme disease slowly expanded from the initial focus, including southward nearly 100 miles (161 km) (12, 17), and became endemic in a contiguous area bordering northern Wisconsin and Minnesota (10).

The disease agent, *B. burgdorferi*, and tick vector, *Ixodes dammini*, have recently expanded farther south from this endemic focus (9, 33, 35). Possible methods of this expansion include movement of infected ticks on white-tailed deer (*Odocoileus virginianus*) (free-ranging deer and fresh deer carcasses moved by hunters), pets, humans, and migratory birds. However, the north-south orientation of the expansion would suggest that either seasonal movement patterns of deer along the Mississippi watershed (16) or southward-migrating birds (26) are involved in the expansion.

A number of tick species parasitize birds (19, 37), and *I. dammini* is commonly found on birds within its known range (2, 7, 41). *B. burgdorferi* was isolated from the liver tissue of a veery (*Catharus fuscescens*) (1) and from *I. dammini* ticks parasitizing a number of species of birds (1, 3, 41). In addition, engorged ticks, including larvae, that had been removed from birds remained infected with *B. burgdorferi* spirochetes following molting, and those spirochetes were infectious to hamsters (*Mesocricetus auratus*) and were indistinguishable from the B31 strain (4). Birds migrating south from and through the endemic focus in Wisconsin and Minnesota have been infested with larval and nymphal *I. dammini*, some of which were infected with *B. burgdorferi* (41). Since transovarial transmission of *B. burgdorferi* in *I. dammini* is generally inefficient (28, 32, 38), the infection of larval ticks removed from birds indicates that birds may serve as reservoir hosts to infect ticks.

The study described here was conducted at the Saint Croix National Riverway in northwestern Wisconsin, in the middle of the Lyme disease-endemic focus, to further evaluate the role of birds as natural reservoir hosts for *B. burgdorferi* and to determine the species of birds involved as maintenance and dissemination hosts.

MATERIALS AND METHODS

Study site and field sampling. The Saint Croix River is a northern tributary of the Mississippi River, and it forms the border between Minnesota and Wisconsin north of St. Paul, Minn. The study site is located along the Wisconsin side of the river within the Saint Croix National Riverway and is described elsewhere (41). Birds were captured with ground-level mist nets. After the species and ages of the birds were determined, they were banded with U.S. Fish and Wildlife Service leg bands, and a small amount of blood (<0.05 ml) was taken from the jugular or brachial vein with a 26- to 27-gauge needle and a 1-ml syringe (39). The birds were released immediately in the vicinity of their capture. Several drops of whole blood from each bird were dispensed directly into 7 ml of Barbour-Stoenner-Kelly (BSK) culture medium in the field. In order to minimize contamination, the skin of each bird was cleansed with 70% alcohol at the site where the needle was inserted for blood specimen collection and the cap of the culture tube was opened only partially and

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TABLE 1. *Borrelia* isolates used in the present study

Isolate	Source	Location
B. burgdorferi WI91-23	*M. melodia*, blood	Wisconsin
B. burgdorferi VLI	*C. fuscescens*, liver	Connecticut
B. burgdorferi B31 (IDS, ATCC 35210)	*I. dammini*	New York
B. burgdorferi CT-1	*I. dammini*	Wisconsin
B. hermsii MAN-1	Human blood	California
B. anserina Es	White leghorn chicken	California

very briefly when dispensing the blood sample into the tube. The inoculated culture tubes were maintained at 27 to 30°C in the field and were transferred to an incubator in the laboratory and maintained at 34°C.

Isolation of spirochetes. Dark-field microscopy was used to screen inoculated cultures weekly for 8 weeks for the presence of spirochetes. Slides were prepared from positive cultures for examination by the fluorescent-antibody test; polyclonal goat anti-*Borrelia* species antibody labeled with fluorescein isothiocyanate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was used for preliminary identification (27). Aliquots of positive cultures were mixed with 30% glycerol and were stored in liquid nitrogen for future testing and reference. Other aliquots were inoculated into additional tubes of BSK medium for passage of isolates. An isolate that underwent a low number of passages (two to six passages) was characterized by antigenic, protein, and molecular procedures, compared with other bacteria, and injected into laboratory animals to determine its infectiousness.

Other bacteria. The *B. burgdorferi* strains used in the present study (Table 1) included VLI (1), B31 (ATCC 35210), and CT-1. *Borrelia anserina* Es was from white leghorn chickens in California (11). *Borrelia hermsii* MAN-1 was obtained from Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Mont. (24). The *Borrelia* strains were virulent and were passaged less than six times in vitro. Spirochetes were grown at 30 or 34°C in BSK medium (5).

Animal inoculation. The cultures used were passaged four times or less in vitro. To examine infectivity, 0.2 ml from the second passage, which contained about 10^4 spirochetes per 0.1 ml, was inoculated subcutaneously and intraperitoneally into each of two adult white-footed mice (*Peromyscus leucopus*) obtained from an uninfected, laboratory-reared colony. Ear biopsy specimens were taken 4 weeks later, and the specimens were placed in BSK I medium and were incubated at 34°C (36). Golden Syrian hamsters (*Mesocricetus auratus*) received 1 ml of the second passage intraperitoneally and were dissected 2 weeks later. The blood, heart, spleen, kidney, liver, and bladder were homogenized in BSK medium, diluted 1:10 in BSK medium supplemented with 0.15% agarose, and incubated at 30°C (23).

IFA. Isolates and *Borrelia* reference strains were tested against a panel of murine monoclonal antibodies (MAbs) by immunofluorescence assay (IFA) (15). The MAbs used included H5332 (OspA), H3TS (OspA), H5TS (OspB), and H9724 (flagellin protein), which were obtained from Rocky Mountain Laboratories. MAb supernatants were used undiluted.

SDS-PAGE. Proteins of the *Borrelia* strains studied were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) by using the buffer systems of Laemmli (25). For electrophoresis, bacteria were harvested by centrifugation at 4°C. The pellet was washed three times with ice-cold 10 mM phosphate-buffered saline (pH 7.2). The final resuspension in 0.0625 M Tris was vortexed, and aliquots were stored at −70°C. The protein content was determined by using a detergent-compatible protein assay (Bio-Rad, Richmond, Calif.). Linear gradient gels (7.5 to 15.0%) (18) were poured at 4°C into a multiple gel caster (Hoefer Scientific Instruments, San Francisco, Calif.) according to the manufacturer's instructions. A 3.75% stacking gel was prepared on the day of electrophoresis. Protein samples were diluted appropriately in sample buffer (0.0625 M Tris, 2% SDS, 15 mM dithiothreitol, 27% sucrose, and 0.002% bromophenol blue) and boiled for 2 min, and a total of 10 μg of protein was loaded per lane. Electrophoresis was carried out at 35 mA of constant current per gel at room temperature. For Western immunoblots, proteins were transferred to Immobilon P membranes (Millipore, Bedford, Mass.) at 1 A of constant current for 30 min by using the buffers of Towbin et al. (40). So that the protein profiles of the *Borrelia* strains could be examined, the gel was stained for 1 h in Coomassie brilliant blue, destained overnight, and then dried.

Western immunoblot. The immunoblot was quenched for 1 h in 0.5% instant dry milk (diluent); this was followed by a 45-min wash with 0.1% Tween 20–Tris-buffered saline (TBS; 20 mM Tris, 500 mM NaCl [pH 7.5]). MAbs H9724 and H3TS were diluted 1:100 and 1:200, respectively. Immunoblots were incubated for 1 h in the MAbs, washed twice in 0.1% Tween 20–TBS, and then incubated for 1 h with goat anti-mouse immunoglobulin G (IgG) conjugated with alkaline phosphatase (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) that had been diluted 1:1,500. The immunoblots were washed twice with 0.1% Tween 20–TBS, twice with TBS, and twice with barbital buffer (150 mM sodium barbital [pH 9.6] with glacial acetic acid). Color development was performed at 22 to 23°C with a developing solution consisting of 300 μg of Nitro Blue Tetrazolium per ml, 150 μg of 5-bromo-4-chloro-3-indolyl phosphate per ml, and 813 μg of $MgCl \cdot 6H_2O$ per ml in barbital buffer.

DNA preparation and techniques. Total (genomic) DNA was isolated from *Borrelia* strains (20). The moles percent G+C content of the *Borrelia* DNA was determined by the method of Marmur and Doty (29). In situ-lysed DNA samples from each *Borrelia* isolate and strain were examined by contour-clamped homogeneous electric field pulsed-field gel electrophoresis (CHEF PFGE) as described previously (21).

RESULTS

Isolation of spirochetes. Thirty-nine adult birds of 17 species were captured at the study site during 14 to 19 June 1991, and their blood specimens were cultured for the presence of spirochetes (Table 2). After 4 weeks, spirochetes were observed in the culture of the blood from an adult song sparrow (*Melospiza melodia*) captured on 15 June 1991. Isolate WI91-23 was preliminarily identified as *Borrelia* spp. by the fluorescent-antibody test. All other cultures were negative for *Borrelia* spp.

Animal infectivity. Spirochetes were isolated in BSK medium from ear tissues taken from two white-footed mice inoculated 4 weeks previously with the isolate WI91-23, and both cultures were positive for *Borrelia* spp. by the fluorescent-antibody test. Spirochetes were also isolated in BSK medium from all of the tissues except the blood of hamsters inoculated 2 weeks previously with this strain.

TABLE 2. Adult birds captured with mist nets at Sandrock Cliffs in the St. Croix National Riverway near Grantsburg, Wis., on 14 to 19 June 1991 and cultured for *B. burgdorferi*

Common name	Scientific name	No. sampled
American goldfinch	*Cardeulis tristis*	8
Red-eyed vireo	*Vireo olivaceus*	5
Song sparrow	*Melospiza melodia*	4[a]
Gray catbird	*Dumetella carolinensis*	4
Ovenbird	*Seiurus aurocapillus*	3
Northern flicker	*Colaptes auratus*	2
Eastern phoebe	*Sayornis phoebe*	2
Golden-winged warbler	*Vermivora chrysoptera*	2
Yellow-billed cuckoo	*Coccyzus americanus*	1
Downy woodpecker	*Picoides pubescens*	1
Ruby-throated hummingbird	*Archilochus colubris*	1
Great-crested flycatcher	*Myiarchus crinitus*	1
Acadian flycatcher	*Empidonax virescens*	1
Indigo bunting	*Passerina cyanea*	1
Nashville warbler	*Vermivora ruficapilla*	1
Common yellowthroat	*Geothlypis trichas*	1
American redstart	*Setophaga ruticilla*	1

[a] Spirochetes isolated from the blood of one song sparrow in BSK culture medium.

Reactivity of WI91-23 to MAbs. Isolate WI91-23 from a song sparrow was identified as *B. burgdorferi* by IFA (Table 3). All the strains of the various *Borrelia* species tested and isolate WI91-23 reacted with MAb H9724 (against the flagellin protein). WI91-23, along with the three other *B. burgdorferi* strains, reacted with MAbs directed against the outer surface proteins of *B. burgdorferi* (H5TS, H5332, and H3TS), whereas *B. hermsii* MAN-1 and *B. anserina* Es did not.

Western immunoblots (Fig. 1) confirmed the IFA results and revealed that the OspA of WI91-23 was similar in size to the OspA of other North American *B. burgdorferi* isolates. The flagellin protein of WI91-23 was also similar in size to that of the other *B. burgdorferi* isolates studied, whereas the flagellin proteins of *B. hermsii* MAN-1 and *B. anserina* Es were smaller (approximately 40 kDa).

Protein profile. SDS-PAGE with a linear gradient gel was used to separate the proteins of the *Borrelia* strains studied. WI91-23 had a protein profile similar to that of *B. burgdorferi* and a protein profile different from those of *B. hermsii* MAN-1 and *B. anserina* Es (Fig. 2). The range in the sizes of the proteins examined was from 10 to 100 kDa. The major proteins of *B. burgdorferi* that we observed included those of 62, 41, 34, and 31 kDa. Although a very weak protein band

TABLE 3. Reactivities of *Borrelia* isolates by IFA to a panel of MAbs[a]

Isolate	MAb reactivity[b]			
	H9724	H5TS	H5332	H3TS
B. burgdorferi WI91-23	+	+	+	+
B. burgdorferi VLI	+	+	+	+
B. burgdorferi B31	+	+	+	+
B. burgdorferi CT-1	+	+	+	+
B. hermsii MAN-1	+	−	−	−
B. anserina Es	+	−	−	−

[a] MAbs H9724 (flagellin protein), H5TS (OspB), H5332 (OspA), and H3TS (OspA).
[b] +, reactive; −, nonreactive.

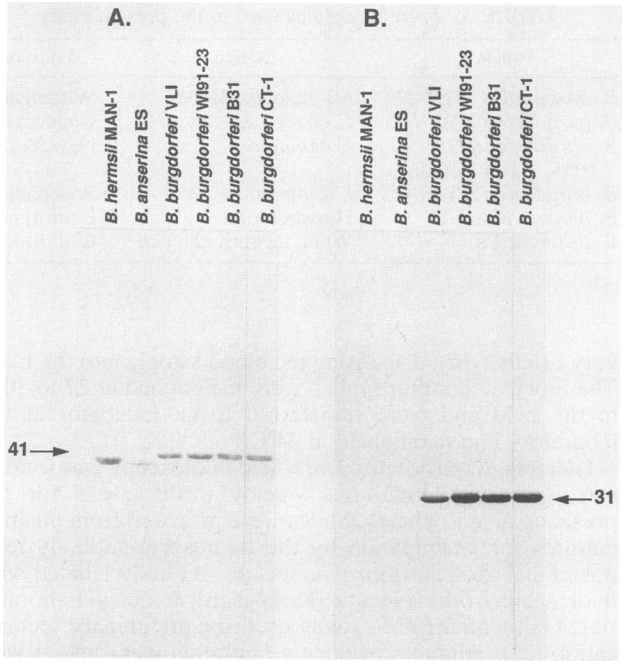

FIG. 1. Western immunoblot of *Borrelia* isolates after probing with MAbs. Arrows indicate the calculated molecular sizes (in kilodaltons) of reactive proteins. (A) Probed with MAb H9724 (flagellin protein); (B) probed with *B. burgdorferi*-species specific MAb H3TS (OspA).

in the appropriate size range of 39 kDa was observed, the identity of this protein is not known at this time. *B. hermsii* MAN-1 had major proteins at 40 and 23 kDa, while *B. anserina* Es had major proteins at 40 and 24.5 kDa.

Characterization of WI91-23 DNA and plasmids. The DNA of *B. burgdorferi* WI91-23 was compared with those of other known *Borrelia* spp. Total (genomic) DNAs were isolated from the test species and strains, and the moles percent G+C contents were determined (Table 4). The melting temperature (T_m) for WI91-23 was determined to be 80.4°C. The T_ms for the other *Borrelia* strains were not found to be significantly different. On the basis of these determinations, the moles percent G+C content of WI91-23 was calculated to be 27.1, which was similar to those calculated for the other *Borrelia* strains studied.

In situ-lysed DNA samples from each *Borrelia* strain in the study were analyzed by CHEF PFGE to determine their plasmid contents (Fig. 3). Isolate WI91-23 contained six plasmids of approximately 60, 40, 32, 29, 26, and 17 kb (Table 5). The 60-kb plasmid appeared to correspond to the linear plasmid that is 49 kb in size in conventional agarose gel electrophoresis and that encodes the OspA and OspB proteins of *B. burgdorferi*. The plasmid profiles of the other *Borrelia* isolates are also indicated in Table 5. The DNA profile of WI91-23 was most similar to that of *B. burgdorferi* and most different from those of *B. anserina* and *B. hermsii*. The plasmid profiles for *B. hermsii* and *B. anserina* were discernibly different from those profiles for the *B. burgdorferi* strains. A large plasmid, approximately 175 kb, was present in *B. hermsii* but was absent from the other *Borrelia* isolates, including isolate WI91-23. The agent of avian borreliosis, *B. anserina*, contained a plasmid of approximately 90 kb in size, but it lacked a 60-kb plasmid which was present in the other *Borrelia* isolates.

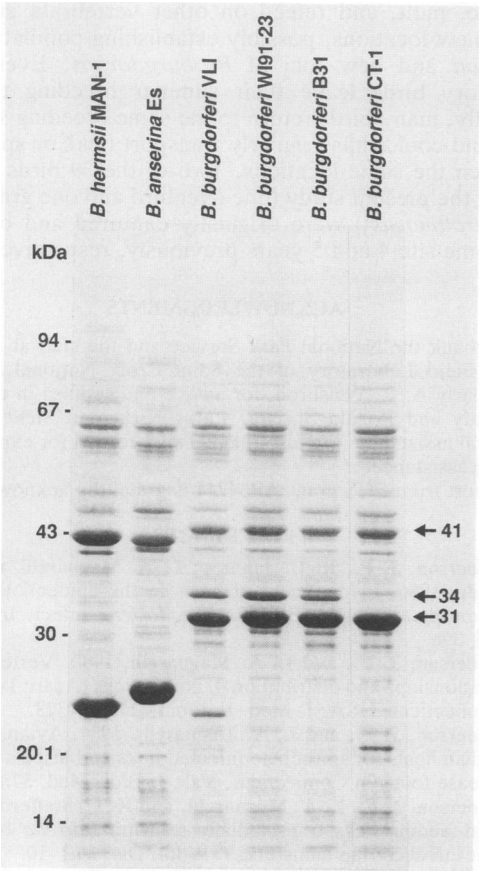

FIG. 2. Coomassie blue-stained 7.5 to 15% linear gradient polyacrylamide gel comparing the protein profile of isolate WI91-23 with those of other *Borrelia* isolates. Molecular size standards are shown on the left. OspA, OspB, and the flagellin protein are indicated by the arrows on the right.

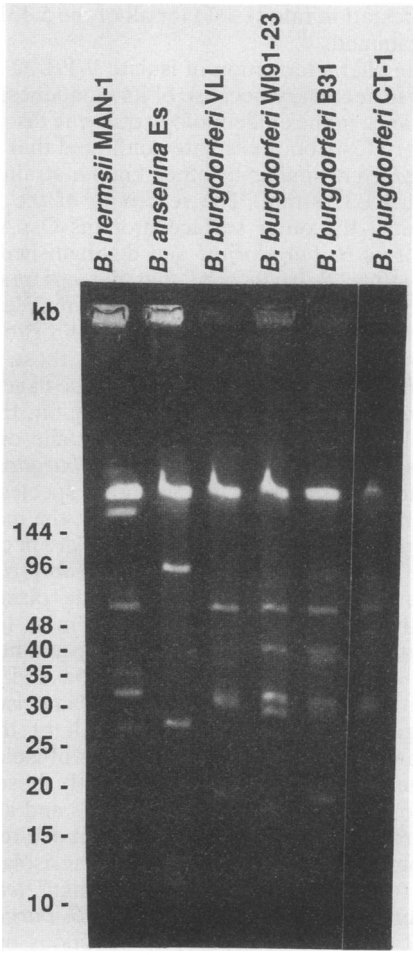

FIG. 3. CHEF PFGE of *Borrelia* DNAs. In situ-lysed DNA samples were separated at 200 V with 5-s pulses for 5 h; this was followed by 1-s pulses for 10 h and then 0.5-s pulses for 2 h. Size markers are indicated on the left.

DISCUSSION

This report contains the first description of a confirmed isolation of *B. burgdorferi* from the blood of a naturally infected bird and the first indication that song sparrows may be implicated as reservoir hosts for Lyme disease. In previous studies, spirochetes initially grew in BSK medium cultures of blood from six different free-ranging species of birds in Connecticut, but the spirochetes did not survive in culture to be identified (3), *B. burgdorferi* spirochetes were isolated from the liver but not from the blood of a veery (1), and *B. burgdorferi* was reisolated from the blood of one mallard duck (*Anas platyrhynchos platyrhynchos*) that had been experimentally inoculated intravenously 7 days previously (8). Immature *I. dammini* ticks have been found on song sparrows in Connecticut (1, 4), in New York (7), and at the study site in the Saint Croix River Valley in Wisconsin (41), but *I. dammini* ticks infected with *B. burgdorferi* were found on song sparrows only at the Wisconsin site (41). The infestation rate of *I. dammini* on the 176 song sparrows examined (1.1%) there in 1988 and 1989 was similar to the

TABLE 4. Moles percent G+C contents of selected *Borrelia* isolates

Isolate	T_m (°C)	Mol% G+C content[a]
B. burgdorferi WI91-23	80.43 ± 0.34	27.1
B. burgdorferi VLI	80.50 ± 0.30	27.1
B. burgdorferi B31	80.27 ± 0.42	26.8
B. burgdorferi CT-1	80.34 ± 0.31	26.9
B. hermsii MAN-1	81.03 ± 0.39	28.6
B. anserina Es	80.70 ± 0.91	27.8

[a] Results are the averages of three experiments and were determined by the method of Marmur and Doty (29), where T_m = 69.3 + [0.41 (mol% G+C content)] when the solvent is 0.2 M Na^+.

TABLE 5. Plasmid profiles of *B. burgdorferi* WI91-23 and other selected *Borrelia* isolates

Isolate	Plasmid size (kb)[a]
B. burgdorferi WI91-23	60, 40, 32, 29, 26, 17
B. burgdorferi VLI	60, 40, 37, 32, 30, 25, 19
B. burgdorferi B31	85, 60, 41, 38, 31, 29, 18
B. burgdorferi CT-1	60, 38, 31, 29, 26, 23, 20
B. hermsii MAN-1	175, 65, 60, 35, 32, 27, 14
B. anserina Es	90, 28, 13, 11

[a] Approximated following CHEF PFGE.

average infestation rate (1.1%) for all of the 5,131 birds of 15 species examined.

Complete characterization of isolate WI91-32 from a song sparrow was necessary because of its uniqueness and potential importance in the epidemiology of Lyme disease. Results of laboratory tests on the isolate confirmed that it is a strain of *B. burgdorferi* similar to other known strains, including the standard B31 strain. The reactivity of the isolate with MAbs against the outer surface proteins OspA and OspB identified it as *B. burgdorferi* and distinguished the isolate from two other *Borrelia* spp. that did not react. Further confirmatory evidence was obtained with Western blots, which showed that the OspA and flagellin proteins of the WI91-23 strain were similar in size to those from other strains of *B. burgdorferi*, but larger than the flagellin proteins from *B. anserina* and *B. hermsii*. In addition, the results of SDS-PAGE revealed that the protein profile of the isolate was similar to the profiles for other *B. burgdorferi* strains and distinct from those for the two other species of *Borrelia* tested. The results of DNA analyses, i.e., the moles percent G+C and plasmid contents, of the isolate are in concordance with those for other *Borrelia* and *B. burgdorferi* strains (21, 22). None of the isolates had the same plasmid profile, although the plasmids of WI91-23 were most like those of other *B. burgdorferi* strains (Table 5). Plasmid heterogeneity among *B. burgdorferi* strains is common and reflects the genetic diversity that exists among strains isolated from different geographical areas (6, 21). All of these results indicate that the antigenic characteristics of isolate WI91-23 from a song sparrow are consistent with those of two *B. burgdorferi* strains from *I. dammini* ticks and a strain from another bird species and are similar to that of a recent human isolate from this endemic focus (21). The isolate was also infectious for two rodent species, including *P. leucopus*, one of the natural reservoir host species for *B. burgdorferi* (13), whereas *B. anserina* is apparently infectious only to birds (1). How this spirochete (*B. burgdorferi*) can exist at the elevated in vivo body temperatures in passerine birds (40 to 41°C) is unknown and probably can be determined only through experimental studies.

The occurrence of spirochetemia in birds further supports previous evidence that they may be reservoir hosts for *B. burgdorferi* (1). The current isolation of *B. burgdorferi* from the blood of a song sparrow combined with the previous results on the infection of larval *I. dammini* ticks removed from song sparrows confirm the reservoir potential of this species. The song sparrow may not be any more important as a reservoir host for *B. burgdorferi* than other avian species, but rather, it is one of many species of birds that nest and forage on the ground and that are natural hosts for the tick vector and spirochete pathogen of Lyme disease. Apparently, however, not all avian species are competent reservoirs for *B. burgdorferi* (30). Birds probably acquire *B. burgdorferi* from infected *I. dammini* nymphs and then subsequently infect larval *I. dammini* that attach later in the summer. The relative importance of birds to the local maintenance and amplification of Lyme disease is unknown. Nevertheless, birds that are summer residents within disease-endemic foci, such as the veery, song sparrow, American robin (*Turdus migratorius*), and ovenbird (*Seiurus aurocapillus*), and those northern species like the gray-cheeked thrush (*Catharus minimus*) and northern waterthrush (*Seiurus noveboracensis*) that pass through disease-endemic foci during migration can carry both the spirochete and infected ticks with them to southern locations during their fall migration. Replete immature ticks that fall to the ground may survive, molt, and refeed on other vertebrate animals at these new locations, possibly establishing populations of *I. dammini* and new foci of *B. burgdorferi*. Even though migratory birds leave their summer breeding territories annually, many birds return to the same breeding sites each year and could thus regularly transport ticks or spirochetes between the same locations. Two of the 39 birds captured during the present study (one ovenbird and one gray catbird [*D. carolinensis*]) were originally captured and banded at this same site 4 and 5 years previously, respectively.

ACKNOWLEDGMENTS

We thank the National Park Service and the staff at the Spring Creek Field Laboratory of the Saint Croix National Riverway, particularly A. R. Weisbrod, for advice and support in conducting the study and Ronald Shriner, Larry Kirk, and Steve Sviat for technical assistance. We also thank Tim Leonard for expert photographic assistance.

Support from PHS grant AR34744 is gratefully acknowledged.

REFERENCES

1. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde.** 1986. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. Infect. Immun. **51:** 394–396.
2. **Anderson, J. F., and L. A. Magnarelli.** 1980. Vertebrate host relationships and distribution of ixodid ticks (Acari: Ixodidae) in Connecticut, USA. J. Med. Entomol. **17:**314–323.
3. **Anderson, J. F., and L. A. Magnarelli.** 1984. Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. Yale J. Biol. Med. **57:**627–641.
4. **Anderson, J. F., L. A. Magnarelli, and K. C. Stafford III.** 1990. Bird-feeding ticks transstadially transmit *Borrelia burgdorferi* that infect Syrian hamsters. J. Wildl. Dis. **26:**1–10.
5. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
6. **Barbour, A. B.** 1988. Plasmid analysis of *Borrelia burgdorferi*, the Lyme disease agent. J. Clin. Microbiol. **26:**475–478.
7. **Battaly, G. R., D. Fish, and R. C. Dowler.** 1987. The seasonal occurrence of *Ixodes dammini* and *Ixodes dentatus* (Acari: Ixodidae) on birds in a Lyme disease endemic area of southeastern New York State. J. N.Y. Entomol. Soc. **95:**461–468.
8. **Burgess, E. C.** 1989. Experimental inoculation of mallard ducks (*Anas platyrhynchos*) with *Borrelia burgdorferi*. J. Wildl. Dis. **25:**99–102.
9. **Callister, S. M., W. A. Agger, R. F. Schell, and J. L. E. Ellingson.** 1988. *Borrelia burgdorferi* infection surrounding La Crosse, Wis. J. Clin. Microbiol. **26:**2632–2636.
10. **Ciesielski, C. A., L. E. Markowitz, R. Horsley, A. W. Hightower, H. Russell, and C. V. Broome.** 1989. Lyme disease surveillance in the United States, 1983–1986. Rev. Infect. Dis. **2**(Suppl. 6)**:**S1435–S1441.
11. **DaMassa, A. J., and H. E. Adler.** 1979. Avian spirochetosis: natural transmission by *Argas* (*Persicargas*) *sanchezi* (Ixodoidea: Argasidae) and existence of different serologic and immunologic types of *Borrelia anserina* in the United States. Am. J. Vet. Res. **40:**154–157.
12. **Davis, J. P., W. L. Schell, T. E. Amundson, M. S. Godsey, A. Spielman, W. Burgdorfer, A. G. Barbour, M. LaVenture, and R. A. Kaslow.** 1984. Lyme disease in Wisconsin: epidemiologic, clinical, serologic, and entomologic findings. Yale J. Biol. Med. **57:**685–696.
13. **Donahue, J. G., J. Piesman, and A. Spielman.** 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hyg. **36:**92–96.
14. **Dryer, R. F., P. G. Goellner, and A. S. Carney.** 1979. Lyme arthritis in Wisconsin. JAMA **241:**498–499.
15. **Gill, J. S., and R. C. Johnson.** 1992. Immunologic methods for the diagnosis of infections by *Borrelia burgdorferi* (Lyme disease), p. 452–458. *In* N. R. Rose, E. C. DeMacario, J. L. Fahey, H. Friedman, and G. M. Penn (ed.), Manual of clinical labora-

tory immunology, 4th ed. American Society for Microbiology, Washington, D.C.
16. **Gladfelter, H. L.** 1978. Movement and home range of deer as determined by radio telemetry. Iowa Wildl. Res. Bull. **23**:1–27.
17. **Godsey, M. S., T. E. Amundson, E. C. Burgess, W. Schell, J. P. Davis, R. Daslow, and R. Edelman.** 1987. Lyme disease ecology in Wisconsin: distribution and host preferences of *Ixodes dammini*, and prevalence of antibody to *Borrelia burgdorferi* in small mammals. Am. J. Trop. Med. Hyg. **37**:180–187.
18. **Hames, B. D., and D. Rickwood.** 1981. Gel electrophoresis of proteins: a practical approach. IRL Press Limited, Oxford.
19. **Hoogstraal, H., and M. N. Kaiser.** 1961. Ticks from European-Asiatic birds migrating through Egypt into Africa. Science **133**:277–278.
20. **Hughes, C. A. N., and R. C. Johnson.** 1990. Methylated DNA in *Borrelia* species. J. Bacteriol. **172**:6602–6604.
21. **Hughes, C. A. N., C. B. Kodner, and R. C. Johnson.** 1992. DNA analysis of *Borrelia burgdorferi* NCH-1, the first northcentral U.S. human Lyme disease isolate. J. Clin. Microbiol. **30**:698–703.
22. **Johnson, R. C., F. W. Hyde, and C. M. Rumpel.** 1984. Taxonomy of the Lyme disease spirochetes. Yale J. Biol. Med. **57**:529–537.
23. **Johnson, R. C., N. Marek, and C. Kodner.** 1984. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin. Microbiol. **20**:1099–1101.
24. **Kurashige, S., M. Bissett, and L. Oshiro.** 1990. Characterization of a tick isolate of *Borrelia burgdorferi* that possesses a major low-molecular-weight surface protein. J. Clin. Microbiol. **28**: 1362–1366.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
26. **Lincoln, F. C., and S. R. Peterson.** 1979. Migration of birds. Fish & Wildlife Service, U.S. Department of the Interior, Circular no. 16. U.S. Department of the Interior, Washington, D.C.
27. **Lord, R. D., J. G. Humphreys, V. R. Lord, R. G. McLean, and C. L. Garland.** 1992. *Borrelia burgdorferi* infection in white-footed mice (*Peromyscus leucopus*) in hemlock (*Tsuga canadensis*) habitat in western Pennsylvania. J. Wildl. Dis. **28**:364–368.
28. **Magnarelli, L. A., J. F. Anderson, and D. Fish.** 1987. Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari: Ixodidae). J. Infect. Dis. **156**:234–236.
29. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
30. **Mather, T. N., S. R. Telford III, A. B. MacLachlan, and A. Spielman.** 1989. Incompetence of catbirds as reservoirs for the Lyme disease spirochete (*Borrelia burgdorferi*). J. Parasitol. **75**:66–69.
31. **Miller, G. L., R. B. Craven, R. E. Bailey, and T. F. Tsai.** 1990. The epidemiology of Lyme disease in the United States, 1987–1988. Lab. Med. **21**:285–289.
32. **Piesman, J., J. G. Donahue, T. N. Mather, and A. Spielman.** 1986. Transovarially acquired Lyme disease spirochetes (*Borrelia burgdorferi*) in field-collected larval *Ixodes dammini* (Acari: Ixodidae). J. Med. Entomol. **23**:291.
33. **Pinger, R. P., and T. Glancy.** 1989. *Ixodes dammini* (Acari: Ixodidae) in Indiana. J. Med. Entomol. **28**:130–131.
34. **Scrimenti, R. J.** 1970. Erythema chronicum migrans. Arch. Dermatol. **102**:104–105.
35. **Siegel, J. P., U. Kitron, and J. K. Bouseman.** 1991. Spatial and temporal distribution of *Ixodes dammini* (Acari: Ixodidae) in a northeastern Illinois State Park. J. Med. Entomol. **28**:101–104.
36. **Sinsky, R. J., and J. Piesman.** 1989. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. J. Clin. Microbiol. **27**:1723–1727.
37. **Sonenshine, D. E., E. L. Atwood, C. M. Clifford, J. T. Lamb, Jr., C. H. Robbins, and D. Bridge.** 1966. Bird ticks as disseminators of wildlife diseases. East. Bird Banding News **29**:78–80.
38. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733–740.
39. **Sudia, W. D., R. D. Lord, and R. O. Hayes.** 1970. Collection and processing of vertebrate specimens for arbovirus studies. Communicable Disease Center, Atlanta.
40. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
41. **Weisbrod, A. R., and R. C. Johnson.** 1989. Lyme disease and migrating birds in the Saint Croix River Valley. Appl. Environ. Microbiol. **55**:1921–1924.