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BIRD-FEEDING TICKS TRANSSTADIALLY TRANSMIT *BORRELIA BURGDORFERI* THAT INFECT SYRIAN HAMSTERS

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ABSTRACT: Bird-feeding *Ixodes dammini* ticks were documented for the first time to successfully molt and transstadially pass *Borrelia burgdorferi* spirochetes that were indistinguishable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis from the type B31 strain. Forty-six of 73 blood-engorged larvae and 50 of 66 fully-fed nymphs, removed from wild-caught birds, successfully molted. Borreliae were isolated from 21 of 78 partially- and fully-fed larvae off birds, including six specimens that molted. Spirochete-positive cultures also were obtained from 35 of 60 partially- and fully-fed nymphs that had fed from birds, including 20 nymphs that molted into adult ticks. Transstadially passed borreliae by bird-feeding larval and nymphal *I. dammini* were infectious to hamsters, leading us to suggest that these ticks are capable of subsequently transmitting infectious spirochetes to mammals, including humans. An isolate of *B. burgdorferi*, recovered from a bird-feeding larval *Ixodes dentatus*, was indistinguishable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis from the B31 strain. This isolate, unlike another from *I. dentatus* off a cottontail rabbit (*Sylvilagus floridanus*), had a protein band with a molecular weight of approximately 31,000 that reacted with murine monoclonal antibodies H3TS and H5332 in western blot analysis. Thus, closely related borreliae are present in both *I. dentatus* and *I. dammini*.

Key words: Lyme disease, *Borrelia burgdorferi*, *Ixodes dammini*, *Ixodes dentatus*, transstadial transmission, hamsters, bird to mammal transmission, experimental study, wild birds.

INTRODUCTION

Wild animals and humans become infected with the Lyme disease agent (Burgdorfer et al., 1982; Steere et al., 1983; Benach et al., 1983) *Borrelia burgdorferi* (Johnson et al., 1984b) following the bite of *Ixodes dammini* ticks in northeastern United States (Burgdorfer et al., 1982). While the white-footed mouse (*Peromyscus leucopus*) has been shown to be an extremely important host for subadult *I. dammini* (Spielman et al., 1979; Piesman and Spielman, 1979; Anderson and Magnarelli, 1980; Carey et al., 1980; Main et al., 1982) and reservoir for *B. burgdorferi* (Anderson et al., 1983, 1985; Bosler et al., 1983; Levine et al., 1985; Loken et al., 1985; Donahue et al., 1987), much less is known about the importance of birds in the epizootiology of Lyme disease. Clearly, birds are parasitized by subadult *I. dammini* (Anderson and Magnarelli, 1984; Anderson et al., 1986; Schulze et al., 1986; Battaly et al., 1987; Anderson, 1988). Furthermore, *B. burgdorferi* has been isolated from the liver of the veery (*Catharus fuscescens*) (Anderson et al., 1986), frequently detected in or isolated from *I. dammini* larvae and nymphs that parasitized birds (Anderson and Magnarelli, 1984; Anderson et al., 1986), detected in blood of seven songbirds from Lyme disease foci (Anderson and Magnarelli, 1984; Schulze et al., 1986), and detected in cloacal material of laboratory inoculated mallard ducks (*Anas platyrhynchos platyrhynchos*) (Burgess, 1989). However, the ability of ticks feeding on birds to successfully molt and transstadially pass spirochetes that are infectious to mammals has not been documented conclusively. Accordingly, we report (1) transstadial transmission of *B. burgdorferi* in *I. dammini* that had fed naturally on wild songbirds, (2) infectiousness in Syrian hamsters of spirochetes isolated from *I. dammini* that had fed to repletion on wild birds and successfully molted in the laboratory, and (3) characterization of major proteins of *B. burgdorferi* isolated from 46 bird-feeding *I. dammini* and one *I. dentatus* specimens that were tested as partially fed ticks or as ticks that had fed fully and molted into the next stage of development.

MATERIALS AND METHODS

Tick collections

Birds were captured in Japanese mist nets in 1988 at two locations within East Haddam, Connecticut (USA), namely Gillette Castle State Park (41°25'30"N, 72°25'30"W) and a 105 ha farm (41°28'25"N, 72°25'30"W) described previously (Anderson et al., 1983). Partially engorged ticks were removed from birds in the field or from those examined in the laboratory. Fully fed ticks were obtained by transferring wild-caught birds to screened cages placed over water. Engorged ticks off each bird were collected from the water daily. All ticks were identified to stage and species.

Birds parasitized by ticks

The following species of birds were parasitized by ticks that were tested for spirochetes. These included the American robin (*Turdus migratorius*), black-and-white warbler (*Mniotilta varia*), black-capped chickadee (*Parus atricapillus*), blue-winged warbler (*Vermivora pinus*), brown-headed cowbird (*Molothrus ater*), Carolina wren (*Thryothorus ludovicianus*), common yellowthroat (*Geothlypis trichas*), gray catbird (*Dumetella carolinensis*), house wren (*Troglodytes aedon*), Louisiana waterthrush (*Seiurus motacilla*), northern cardinal (*Cardinalis cardinalis*), northern mockingbird (*Mimus polyglottos*), ovenbird (*Seiurus aurocapillus*), song sparrow (*Melospiza melodia*), veery (*Catharus fuscescens*), white-eyed vireo (*Vireo griseus*), white-throated sparrow (*Zonotrichia albicollis*), wood thrush (*Hylocichla mustelina*), and yellow warbler (*Dendroica petechia*).

Rearing of fully fed ticks

Engorged larvae and nymphs were placed in vials containing moistened plaster of paris and kept at 100% relative humidity at 25 C. Ticks were maintained under these conditions for 1 to 7 wk after molting, or until they died.

Isolation of borreliae from ticks

Attempts were made to isolate borreliae from the ticks off birds. These specimens included 39 *I. dammini* fully-fed larvae that molted into nymphs, 51 fully-fed *I. dammini* nymphs that molted into adults, 48 and 17 partially-fed larval *I. dammini* and *I. dentatus*, respectively, 27 and one partially-fed nymphal *I. dammini* and *I. dentatus*, respectively, and six partially-fed larval *Haemaphysalis leporispalustris*. All ticks were surface-cleansed with 70% isopropyl alcohol prior to dissection. Nymphal or adult tick tissues were placed into duplicate 7 ml tubes of Barbour-Stoenner-Kelly medium (Barbour, 1984) containing 0.15% agarose (SeaKem, LE; FMC Corporation, Marine Colloids Division, Rockland, Maine 04841, USA), 0.023% L-cysteine hydrochloride, 0.015% DL-dithiothreitol, and 0.002% superoxide dismustase (Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri 63178, USA) (Johnson et al., 1984a). Rifampicin (50 μg/ml) (Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri 63178, USA) was added to one of each pair of tubes (Johnson et al., 1984c). Tissues from individual larval ticks were placed either into medium with rifampicin or into medium without this antibiotic. Cultures were maintained at 31 C for 1 to 6 wk prior to their examination for borreliae by dark-field microscopy.

Experimental infection of Syrian hamsters

Isolates of *B. burgdorferi* (number of cells = 1×10^8) were inoculated intraperitoneally into each of three laboratory reared Syrian hamsters. Inocula were prepared from passages one or two of each of three isolates from *I. dammini* nymphs that had fed as larvae on American robins and of each of three isolates from recently molted *I. dammini* adults that had fed as nymphs on an American robin, northern cardinal or brown-headed cowbird. Attempts were made to isolate *B. burgdorferi* from tissues of spleens, kidneys and bladders of the 18 Syrian hamsters 14 or 20 days postinoculation (Johnson et al., 1984a; Anderson et al., 1985; Schwan et al., 1988).

SDS-PAGE analysis

Isolates from 29 partially-fed *I. dammini* and one *I. dentatus*, 17 fully-fed *I. dammini* that had molted into the next stage, and 16 Syrian hamsters that had been inoculated with *B. burgdorferi* cultured originally from *I. dammini* were compared with the type B31 *B. burgdorferi* strain (Burgdorfer et al., 1982) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Barbour et al., 1983). A SE 600 vertical gel unit (Hoefer Scientific Instruments, San Francisco, California 94107, USA) was used to separate protein bands of whole cell lysates that were prepared from each isolate by procedures described previously (Barbour et al., 1983). Protein bands were stained with Coomassie brilliant blue R-250 or silver stain (Tsai and Frasch, 1982).

Western blot analysis

Proteins of 47 isolates were separated by SDS-PAGE and transferred to nitrocellulose paper (Towbin et al., 1979). Blocking solution (Anderson et al., 1989) was used to saturate the paper prior to its submersion for 2 hr in a 1:100 dilution of murine sera containing monoclonal antibodies H5332, H3TS, H6831, or H9724 (A.

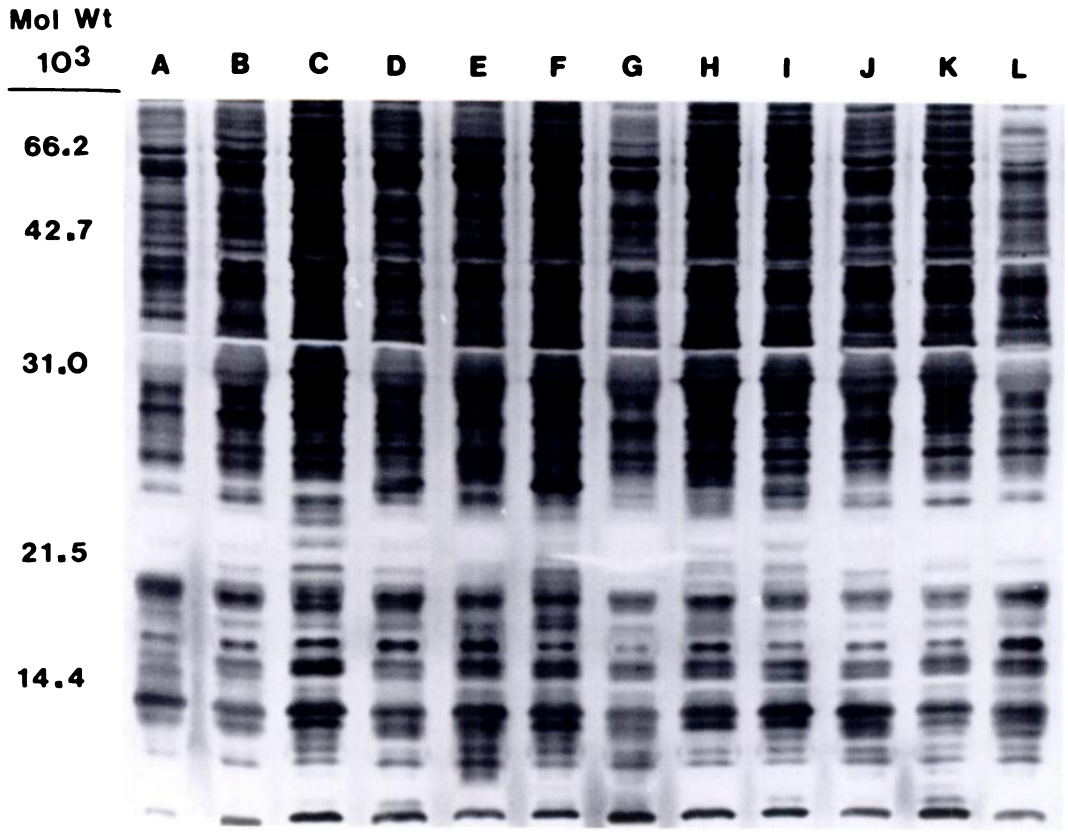

FIGURE 1. Whole-cell lysates of silver-stained *Borrelia burgdorferi* isolates from bird-feeding *Ixodes dammini* compared with the B31 strain. Lanes: A, female adult that molted from a nymph off a wood thrush (strain 27291); B, female adult that molted from a nymph off a brown-headed cowbird (strain 27051); C, larva off a house wren (strain 27620); D, nymph that molted from a larva off American robin number 19 (strain 27078); E, nymph that molted from a larva off American robin number 18 (strain 27059); F, nymph that molted from a larva off American robin number 17 (strain 26278); G, nymph off a yellow warbler (strain 26689); H, nymph off a white-throated sparrow (strain 26103); I, nymph off a Carolina wren (strain 26546); J, nymph off a blue-winged warbler (strain 26376); K, nymph off a black-and-white warbler (strain 26695); L, B31 strain.

G. Barbour of the Department of Microbiology and Medicine, University of Texas Health Science Center, San Antonio, Texas 78284, USA provided the monoclonal antibodies) which react with protein bands of *B. burgdorferi* with approximate molecular weights of 31,000, 31,000, 34,000, and 41,000, respectively (Barbour et al., 1983, 1985, 1986). After a thorough wash in blocking solution, the paper was incubated for 2 hr in a solution of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG, Tago, Inc., Burlingame, California 94010, USA) diluted 1:500 in tris-buffered saline and washed in tris-buffered saline with 0.5% tween-20. Horseradish peroxidase color development reagent (Bio-Rad Laboratories, Rockville Centre, New York 11571, USA) was used to stain attached antibody to specific protein bands. Isolates from bird-feeding ticks were compared with the type strain (B31) of *B. burgdorferi* from *I. dammini* (Burgdorfer et al., 1982) and to strain 19941 cultured from *I. dentatus* that had been removed from a cottontail rabbit (*Sylvilagus floridanus*) (Anderson et al., 1989).

RESULTS

Molting of fully fed *I. dammini*

Forty-six of 73 (63%) blood-engorged larvae off seven species of birds (gray catbird, veery, house wren, black-capped chickadee, American robin, ovenbird, and wood thrush) molted into nymphs; 50 of

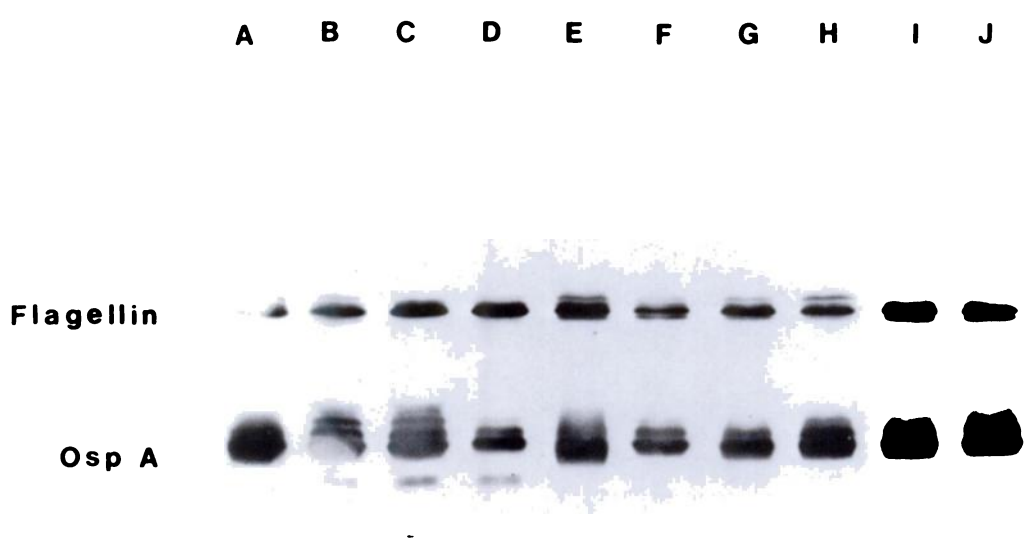

FIGURE 2. Western blot of *Borrelia burgdorferi* isolates from bird-feeding *Ixodes dammini* compared with the B31 strain. The upper protein band (flagellin) was tested against antibody H9724 and the lower protein band (OspA) was tested against antibody H3TS. Lanes: A, B31 strain; B, nymph off white-throated sparrow number 9 (strain 26102); C, nymph off white-throated sparrow number 9 (strain 26105); D, nymph off American robin number 17 (strain 26278); E, nymph off a black-and-white warbler (strain 26375); F, nymph off Carolina wren number 1 (strain 26546); G, nymph off Carolina wren number 1 (strain 26548); H, nymph off American robin number 18 (strain 26639); I, nymph off American robin number 18 (strain 26642); J, nymph off American robin number 18 (strain 26644).

66 (76%) fully-fed nymphs off nine species of birds (gray catbird, veery, Louisiana waterthrush, northern cardinal, American robin, song sparrow, wood thrush, brown-headed cowbird, and white-throated sparrow) molted into males and females.

Borreliae isolated from *I. dammini*

Borreliae were recovered from 21 of 78 (27%) larvae that had fed on birds (an additional nine cultures were contaminated). Seven of eight specimens feeding on American robins in the spring and 14 of 16 larvae off a house wren in the fall were infected with spirochetes. Five of these isolates were from nymphs that had molted after having fully engorged as larvae on three different robins. One isolate was from an adult that had fed as a larva on a house wren and subsequently on a laboratory-bred white-footed mouse as a nymph. Spirochetes were not isolated from larvae removed from an ovenbird ($n = 2$ ticks tested) and a wood thrush ($n = 1$) in the spring, or from larvae feeding on gray catbirds ($n = 40$), black-capped chickadees ($n = 8$), a northern mockingbird ($n = 1$), veery ($n = 1$), or white-eyed vireo ($n = 1$) in the fall.

Borrelia burgdorferi was identified in cultures of 35 of 60 (58%) *I. dammini* specimens that had fed as nymphs on birds. Eighteen other cultures were contaminat-

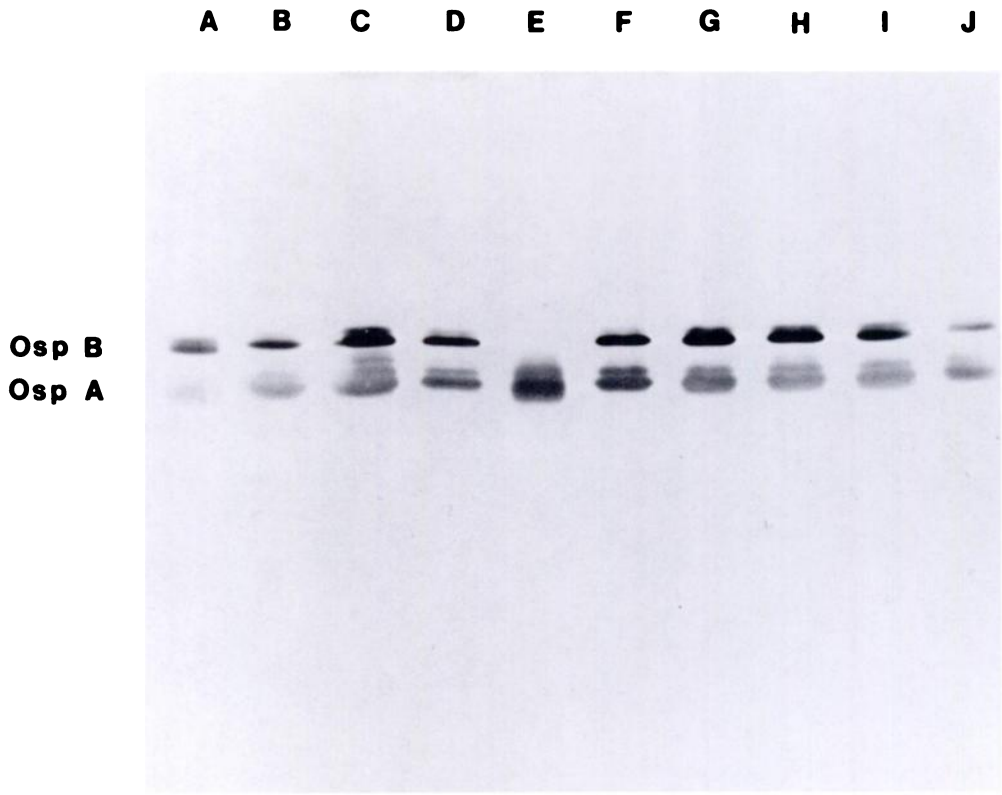

FIGURE 3. Western blot of *Borrelia burgdorferi* isolates from bird-feeding *Ixodes dammini* compared with the B31 strain. The upper protein band (OspB) was tested against antibody H6831 and the lower protein band (OspA) was tested against antibody H5332. Lane identification is the same as in Figure 2.

ed. Twenty-four of the nymphs had fed on four American robins. Cultures also were positive for nymphs that had fed on a black-and-white warbler, blue-winged warbler, Carolina wren, northern cardinal, brown-headed cowbird, white-throated sparrow, yellow warbler and two wood thrushes. Twenty of these isolates were from adult ticks which had fed as nymphs either on a northern cardinal, brown-headed cowbird, American robin or wood thrush.

Tissues from the forty-one positive ticks tested as nymphs or as adults had been placed in duplicate tubes of medium with and without antibiotics. Tissues from 31 ticks yielded spirochetes in both culture tubes. Of the remaining ten isolates two were obtained only in medium without antibiotics, and eight were cultured only in the medium containing rifampicin. Eight of the cultures made directly from tissues of larval ticks were in media without addition of rifampicin; the remaining seven cultures were in medium containing the antibiotic.

The 47 bird-feeding *I. dammini* isolates tested by SDS-PAGE were similar to the original B31 strain (note in Fig. 1 the similarity of protein bands of 11 isolates with the B31 strain, although some variation in bands is evident at molecular weights of about 23,200 to 27,200). All isolates and the B31 strain reacted with monoclonal antibodies H9724, H5332, and H3TS in western blots as illustrated with nine isolates and the B31 strain in Figures 2, 3. Three-fourths of the isolates and the B31

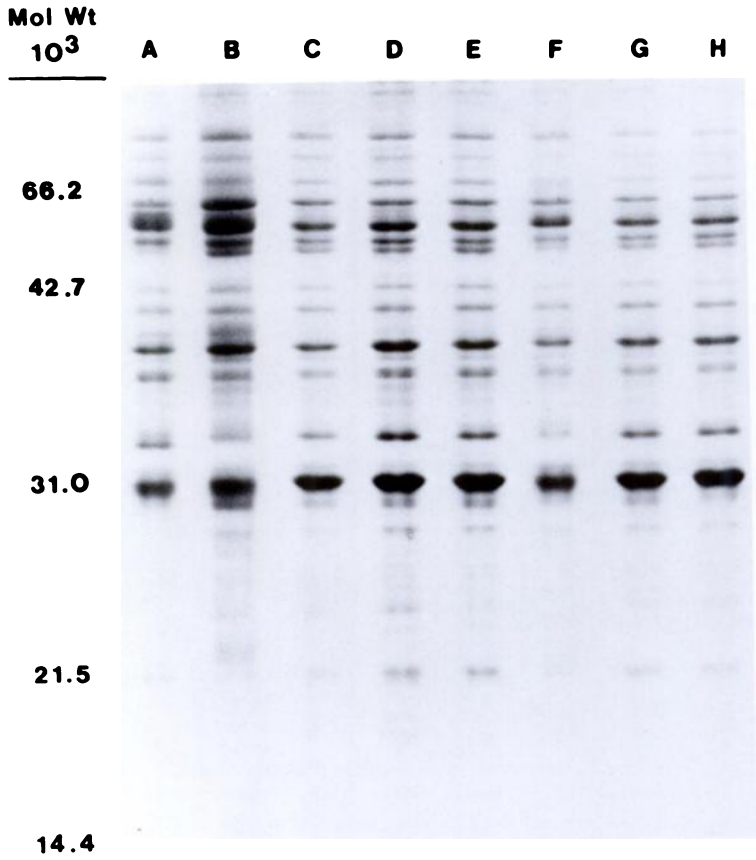

FIGURE 4. Coomassie brilliant blue-stained proteins of whole-cell lysates of two isolates of *Borrelia burgdorferi* from *Ixodes dammini* that molted after feeding on birds compared with reisolates from inoculated Syrian hamsters and with strain B31. Lanes: A, B31 strain; B, nymph that molted from a larva off American robin number 18 (strain 27059); C, bladder from hamster number 1 inoculated with borreliae from tick identified in lane B; D, spleen from hamster number 2 inoculated with borreliae from tick identified in lane B; E, spleen from hamster number 3 inoculated with borreliae from tick identified in lane B; F, female adult that molted from a nymph off a brown-headed cowbird (strain 27051); G, spleen from hamster number 4 inoculated with borreliae from tick identified in lane F; H, spleen from hamster number 5 inoculated with borreliae from tick identified in lane F.

strain reacted with antibody H6831 (note in Fig. 3 that all isolates except the one in lane E reacted with this antibody).

Infectivity of transstadially transmitted borreliae to hamsters

Borrelia burgdorferi was reisolated from tissues of spleens, kidneys or bladders of 16 of 18 Syrian hamsters injected with isolates from three unfed nymphs that had fed previously as larvae on American robins and with isolates from three unfed adult ticks that previously had fed as nymphs on an American robin, a northern cardinal, or a brown-headed cowbird. The isolates from hamsters were indistinguishable from the original inoculum by SDS-PAGE (Fig. 4).

Isolation attempts from *Ixodes dentatus* and *Haemaphysalis leporispalustris*

Borreliae were isolated from one partially-fed *I. dentatus* larva off an American robin. This isolate was indistinguishable from the B31 strain of *B. burgdorferi* by SDS-PAGE and differed in western blots

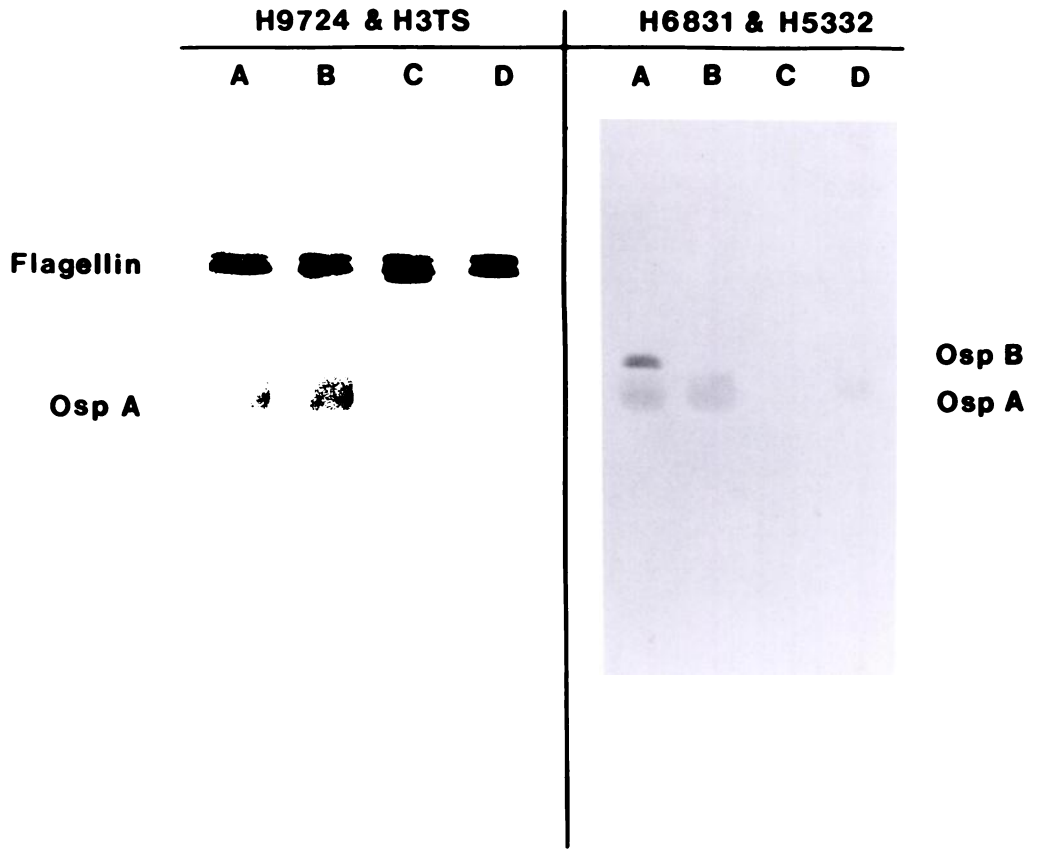

FIGURE 5. Western blot of whole-cell lysates of *Borrelia burgdorferi*. In the photo on the left, the upper protein band (flagellin) was tested against antibody H9724 and the lower protein band (OspA) was tested against antibody H3TS. In the photo on the right, the upper protein band (OspB) was tested against antibody H6831 and the lower protein band (OspA) was tested against antibody H5332. Lanes: A, B31 strain; B, larval *Ixodes dentatus* off an American robin number 20 (strain 26861); C, larval *I. dentatus* off a cottontail rabbit (strain 19941); D, nymphal *Ixodes dammini* that molted from a larva off American robin number 19 (strain 27125).

only by not reacting with antibody H6831 (Fig. 5). By binding with antibodies H5332 and H3TS, the isolate from the bird-feeding *I. dentatus* differed from strain 19941, cultured from *I. dentatus* off a cottontail rabbit (Fig. 5). There were no isolates from nine larvae removed from other American robins (seven additional cultures were contaminated). Likewise, no borreliae were isolated from six larval *H. leporispalustris* collected from a common yellowthroat.

DISCUSSION

We document for the first time that bird-feeding *I. dammini*, like those that feed on rodents, successfully molt and transstadially pass borreliae that are indistinguishable from the original B31 strain of *B. burgdorferi*. Protein bands were similar by SDS-PAGE; flagellin proteins (molecular weight = 41,000) reacted with antibody H9724, identifying the spirochetes as belonging to the genus *Borrelia* (Barbour et al., 1986). Similar to isolates of *B. burgdorferi* recovered from humans and rodents in North America, borreliae from ticks off birds had OspA proteins (molecular weight = 31,000) that invariably reacted with antibodies H5332 and H3TS, and OspB proteins (molecular weight =

34,000) that varied in their reactions with antibody H6831 (Barbour et al., 1985). The demonstrated infectiousness of these borreliae to Syrian hamsters suggests to us that spirochetes are transferred between rodents and birds by subadult *I. dammini* and that ticks on birds are capable of subsequently transmitting infectious spirochetes to humans or other mammalian hosts.

The transstadial passage of borreliae by bird-feeding *I. dammini* supports previous conclusions (Anderson and Magnarelli, 1984; Anderson et al., 1986; Schulze et al., 1986; Battaly et al., 1987; Anderson, 1988) that birds are important in the epizootiology of Lyme disease. During their northward migration in the spring and their flight to southern latitudes in the fall, birds may disperse fully-fed and normally developing infected or noninfected *I. dammini* into new localities. These introductions may establish new foci for the tick and spirochete. Alternatively, the introductions may be ephemeral and occasionally expose people, who reside outside the tick's established geographic range, to tick bites. Also, nesting birds may return ticks that are fully fed to their native forested areas, thereby replenishing local tick populations.

The isolation of *B. burgdorferi* from 21 larvae off three American robins and one house wren suggests to us that these birds are competent reservoirs, although transovarial transmission of spirochetes in ticks cannot be totally ruled out (Piesman et al., 1986; Magnarelli et al., 1987). Previously, borreliae were detected in the blood of an American robin and other song birds (Anderson and Magnarelli, 1984; Schulze et al., 1986), and *B. burgdorferi* even isolated and characterized from a veery (Anderson et al., 1986). However, not all birds are equally important. Some species are infrequently parasitized by *I. dammini* (Anderson and Magnarelli, 1984); others, such as gray catbirds, appear to be rarely spirochetemic (Anderson and Magnarelli, 1984; Mather et al., 1989). Nonetheless, some species of birds are competent hosts for relatively large numbers of juvenile *I. dammini*, and some are competent reservoirs for *B. burgdorferi* as well. Persons considering controlling Lyme disease need to consider both mammals and birds as hosts for the tick and spirochete.

Our isolation of *B. burgdorferi* from *I. dentatus* off an American robin and demonstration that this spirochete is indistinguishable, except for its nonreactivity with monoclonal antibody H6831, from the B31 strain documents for the first time that closely related borreliae occur in both *I. dentatus* and *I. dammini*. Inasmuch as *I. dentatus* feeds on cottontail rabbits (*Sylvilagus floridanus*) that carry distinctly different *B. burgdorferi* (Anderson et al., 1989) and has been shown to transmit a strain similar to B31 (Telford and Spielman, 1989), the role of these ticks in vectoring *B. burgdorferi* variants to rabbits, birds and possibly humans needs clarification.

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BOOK REVIEW . . .

Heartwater: A review, E. Camus and N. Barre. Office International des Epizooties, 12, rue die Prony, 75017 Paris, France. 1988. 147 pp. FF100.

This book is a much needed review of information on heartwater disease that is caused by the rickettsia, *Cowdria ruminantium*. The review is timely because several laboratories throughout the world have joined with the South Africans in researching this organism. The recent discovery of heartwater in the Antilles poses a problem of possible spread to the American continent. The material for this review was compiled in 1982 with cooperation from laboratories in Utrecht, the Netherlands and Onderstepoort, Republic of South Africa, and was translated into English in 1986 by Pamela Oberem, Veterinary Research Institute at Onderstepoort. The review includes both referenced material and personal communications from researchers throughout the world.

The review is divided into five chapters that cover general information, etiology, epidemiology, pathology and diagnosis. Along with current information on heartwater, the review provides a critical assessment of research needs and priorities and highlights areas of research that are important to understanding the epidemiology of heartwater. Specific gaps in our current knowledge about this disease are clearly identified. Since this review was compiled the South Africans have successfully propagated *C. ruminantium* in cell culture and have shared this technology via an international conference in 1986. The culture of *C. ruminantium* has led to studies on the molecular biology of this organism now underway in several laboratories. Also, much of the developmental cycle of the organism in the tick vector is now known.

The review provides an excellent reference book on heartwater for professors and researchers and is accompanied by a thorough review of the literature up to 1982. The material is organized efficiently for easy access and provides a good, basic review of this disease.

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