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Involvement of Birds in the Epidemiology of the Lyme Disease Agent *Borrelia burgdorferi*

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***Borrelia burgdorferi*, the causative agent of Lyme disease, was isolated from the liver of a passerine bird, *Catharus fuscescens* (veery), and from larval *Ixodes dammini* (tick) feeding on *Pheucticus ludovicianus* (rose-breasted grosbeak) and *Geothlypis trichas* (common yellowthroat). In indirect immunofluorescence antibody tests, isolates reacted with polyclonal and monoclonal (H5332) antibodies. Studies on the DNA composition of the veery liver isolate and the strain cultured from an *I. dammini* larva indicated that both were *B. burgdorferi* and not *Borrelia anserina* or *Borrelia hermsii*. The veery liver isolate infected hamsters and a chick. In contrast, *B. anserina* infected chicks but not hamsters. *B. burgdorferi* is unique among *Borrelia* spp. in being infectious to both mammals and birds. We suggest that the cosmopolitan distribution of *B. burgdorferi* may be caused by long-distance dispersal of infected birds that serve as hosts for ticks.**

In the northeastern United States, *Borrelia burgdorferi* (21) is transmitted primarily by *Ixodes dammini* (15), a tick that parasitizes a variety of mammals and birds (3, 5, 18, 30, 33). This spirochete has been isolated, cultured, and identified from *I. dammini* (4–6, 15, 22, 25), *Ixodes pacificus* (17), *Dermacentor variabilis* (2), humans (12, 37), and wild animals (2, 4–6, 13, 25, 27) in the United States. In Europe, it has been cultured from *Ixodes ricinus* (1, 10, 16) and from humans (7, 8, 31, 32). Spirochetes resembling *B. burgdorferi* also have been detected in two other species of ticks, two insects, and an arthritic dog (5, 26, 35). In addition, serological analyses have provided supportive evidence of this spirochete in wild and domestic mammals (24, 28, 29). Several birds captured in a Lyme disease focus were spirochetemic, but these bacteria were not cultured or identified (5). We now report the first isolation of *B. burgdorferi* from a bird and from larval *I. dammini* feeding on other passerines. We also compare the infectivity of the bird isolate in hamsters and chicks with *Borrelia anserina*, the causative agent of avian borreliosis (23).

MATERIALS AND METHODS

Birds were captured in Japanese mist nets from 13 July through 24 August 1984 in a Lyme disease focus in East Haddam, Conn. (5). Ticks were removed from the heads and necks of birds and were identified.

Attempts to isolate *Borrelia* spp. from birds were made by inoculating 2 drops (0.02 ml) of heparinized blood into 7 to 8 ml of Barbour-Stoenner-Kelly culture medium (9). Tissues from the kidneys, spleens, and livers were aseptically excised, and each was triturated in 2 ml of medium. A 0.1-ml inoculum was then added to 7 ml of medium, and preparations were incubated at 34°C for at least 21 days before examination for spirochetes by dark-field microscopy.

I. dammini ticks (n = 169) were tested for spirochetes by aseptically removing their midguts and either inoculating them into Barbour-Stoenner-Kelly medium or examining these tissues by direct fluorescent antibody staining (15).

Spirochete isolates were characterized serologically with polyclonal [Swiss mouse antisera to *B. burgdorferi* isolate Ct 2591 (6)] and murine monoclonal antibodies (H5332) directed against the 31-kilodalton surface protein of *B. burgdorferi* B31 strain (11) by using the indirect immunofluorescence antibody assay. The DNA relatedness of isolates from a bird and larval *I. dammini* to other *Borrelia* spp. was determined with the nitrocellulose filter technique (19).

The infectious nature of the isolate from the bird was examined by injecting ca. 10^8 spirochetes intraperitoneally into each of three Syrian hamsters, *Mesocricetus auratus* (20), and into each of three 1-day-old chicks, *Gallus gallus*. In parallel experiments with ca. 10^8 microorganisms, we inoculated *B. anserina* intraperitoneally into both hamsters and chicks. Blood and tissues of spleens and kidneys were aseptically removed from each test animal 14 days after inoculation and inoculated into a Barbour-Stoenner-Kelly medium for the recovery of spirochetes (20).

RESULTS

Subadult *I. dammini* parasitized 44 (71%) of the 62 birds captured (Table 1). *Borrelia* spp. were observed by direct fluorescent antibody staining in larval and nymphal *I. dammini* attached to 8 of the 18 species of birds examined. Spirochetes were cultured from seven larvae removed from a common yellowthroat, *Geothlypis trichas*, and from one larva feeding on a rose-breasted grosbeak, *Pheucticus ludovicianus*.

Spirochetes were isolated from the liver (but not from the kidney, spleen, or blood) of a veery, *Catharus fuscescens*, captured on 15 August. This bird was parasitized by eight immature *I. dammini*, including one nymph and four larvae infected with spirochetes. No spirochetes were isolated from samples of blood (n = 32), kidneys (n = 17), spleens (n = 11), or livers (n = 12) of 40 other birds (14 species).

The veery liver isolate (VLI) and the eight larval *I. dammini* isolates (ticks removed from a common yellowthroat and a rose-breasted grosbeak) reacted with the monoclonal antibody H5332. All isolates also were serologically indistinguishable when tested against Swiss mouse antiserum to *B. burgdorferi*. Neither *B. anserina* nor *Borrelia hermsii*, the etiological agent of tick-borne relapsing fever in the western United States (23), reacted with H5332 antibody. The DNA studies, conducted at 60°C (intermediate

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TABLE 1. Prevalence of spirochete-infected *I. dammini* infesting birds[a]

Common name	Scientific name	No. of birds infested/no. of birds examined	Mean no. of ticks/bird ± SD or no. of ticks/single bird		No. of spirochete-positive ticks/no. of ticks examined	
			Larva	Nymph	Larva	Nymph
Blue-winged warbler	*Vermivora pinus*	5/8	0.5 ± 1.1	0.7 ± 1.2	0/3	0/1
Common yellowthroat	*Geothlypis trichas*	6/9	3.3 ± 5.8	1.2 ± 1.7	10/29	1/2
Eastern phoebe	*Sayornis phoebe*	1/4	0.2 ± 0.5	0	ND[b]	ND
Gray catbird	*Dumetella carolinensis*	11/13	2.2 ± 3.2	0.5 ± 1.0	2/20	0/2
Hooded warbler	*Wilsonia citrina*	1/1	0	2	ND	0/1
House wren	*Troglodytes aedon*	2/2	12 ± 11.3	0.5 ± 0.7	6/21	0/1
Least flycatcher	*Empidonax minimus*	0/3	0	0	ND	ND
Northern cardinal	*Cardinalis cardinalis*	2/2	0.5 ± 0.7	6.5 ± 7.8	ND	6/9
Northern water thrush	*Seiurus noveboracensis*	1/1	5	0	ND	ND
Ovenbird	*Seiurus aurocapillus*	1/2	1.5 ± 2.1	0.5 ± 0.7	0/2	1/1
Rose-breasted grosbeak	*Pheucticus ludovicianus*	4/4	0.5 ± 1.0	3.7 ± 4.1	1/7	0/5
Song sparrow	*Melospiza melodia*	1/2	0	0.5 ± 0.7	ND	0/1
Swamp sparrow	*Melospiza georgiana*	2/3	6.3 ± 11.0	1.3 ± 1.5	0/19	0/3
Veery	*Catharus fuscescens*	2/2	3.0 ± 4.2	1.5 ± 0.7	4/6	2/2
White-eyed vireo	*Vireo griseus*	2/2	0	2.3 ± 2.1	ND	ND
Wood thrush	*Hylocichla mustelina*	1/1	0	2	ND	ND
Worm-eating warbler	*Helmitheros vermivorus*	1/1	3	1	ND	ND
Yellow warbler	*Dendroica petechia*	1/2	15.5 ± 21.9	2.0 ± 2.8	3/30	1/4

[a] Captured in East Haddam, Conn., in July and August 1984.
[b] ND, Not done.

stringency temperature for reassociation), indicated that the VLI and *I. dammini* isolates were *B. burgdorferi*, with relatedness values of 46 and 68%, respectively. In addition, the DNA hybrid of *B. burgdorferi* and VLI showed minimal sequence divergence (0.5%), verifying their relatedness. The DNA of *B. anserina* and *B. hermsii* showed relatedness of 35 and 59% (sequence divergence of 18%) to *B. burgdorferi*. These studies show that *B. burgdorferi* is distinct from the two other *Borrelia* spp. (14, 19).

The VLI was isolated from kidney and spleen tissues of three inoculated hamsters and one inoculated chick; the cultures containing tissues of the other chicks were contaminated. *B. anserina* infected the three test chicks, but these spirochetes were not recovered from spleen or kidney tissues of the three inoculated hamsters.

DISCUSSION

Our recovery of *B. burgdorferi* from the liver of a veery is the first isolation of this pathogen from a bird. While *Borrelia* spp. were previously demonstrated in (but not cultured from) birds captured in a Lyme disease focus (5), genetic or serologic analyses were not performed to distinguish the spirochetes from those of the *Borrelia* type species, *B. anserina*, which also infects birds (23). These two *Borrelia* spp. can be distinguished in indirect immunofluorescent antibody tests with the use of specific monoclonal antibodies (11) or by DNA relatedness studies (14, 19, 21, 34).

The infectious nature of *B. burgdorferi* in both mammals and birds was confirmed by recovering the VLI from laboratory-inoculated hamsters and chicks. In contrast, *B. anserina* infected only chicks as previously reported (23). Since *I. dammini* subadults feed extensively on birds and mammals (3, 5, 18, 30, 36, 38), and since *B. burgdorferi* has been isolated from larvae and nymphs feeding on these hosts (5, 6, 25) and has been isolated directly from blood or tissues of mammals (2, 4–6, 13, 25, 27) and now from a wild bird, both groups of vertebrates would appear to be suitable reservoirs for this globally distributed spirochete (33).

B. burgdorferi is unique among the *Borrelia* spp. in being infectious to both birds and mammals. Birds seem to be a natural means of disseminating this spirochete within and among continents. Only two other *Borrelia* spp. are cosmopolitan: *B. anserina* and *B. recurrentis* (23). The latter species, not presently known to occur in North America, infects humans and is vectored by the body louse, *Pediculus humanus humanus*. The mobility of humans carrying lice accounts for the broad distribution of *B. recurrentis*. The long-distance dispersal of birds is likely responsible for the worldwide distribution of *B. anserina*. The remaining 17 *Borrelia* spp. infect only mammals and their tick vectors and are restricted to either Old World or New World habitats (23).

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