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Role of Bird Migration in the Long-Distance Dispersal of *Ixodes dammini*, the Vector of Lyme Disease

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To evaluate the role of migratory birds in the long-distance dispersal of *Ixodes dammini* ticks and in the spread of Lyme disease, a 6-year study of migrating birds to an offshore New England island was conducted during 1989–1994. *I. dammini* are not endemic on this island, therefore allowing assessment of long-distance tick dispersal rather than local infestation. Of 11,324 spring migrants examined, 1.2% were infested with *I. dammini*. Of 8607 fall migrants examined, 0.2% were infested. Of nymphal ticks examined, 20% were infected with *Borrelia burgdorferi*. OspB DNA sequencing of 6 *B. burgdorferi* isolates was identical to sequences of 2 strains common in coastal Maine. It is evident that bird migration allows for long-distance dispersal of *I. dammini* from areas where they are endemic to areas where they are not and that a few bird species account for the majority of tick dispersal. The likelihood of establishment of enzootic Lyme disease by this mechanism is discussed.

Since the first description of Lyme disease in the United States, state public health departments and the Centers for Disease Control and Prevention have documented a progressive rise in reported cases over an enlarging geographic area that encompasses much of the northeastern United States [1]. Although increased recognition of the disease has contributed to increased reporting of cases, entomologic data suggest an expansion of the range of its tick vector, *Ixodes dammini* [2]. However, the means of tick dispersal to new areas has received little attention. Passive transport of ticks by large mammal hosts, such as deer, fails to explain the extensive and discontinuous distribution of *I. dammini*.

Juvenile *I. dammini* ticks commonly feed on birds, and passive dispersal during migration has been suggested [3–5]. However, tick infestation of birds has been documented only in sites where *I. dammini* are endemic, making conclusions regarding long-distance dispersal of ticks by birds conjectural. In this study, we evaluate the role of migrating birds in the dispersal of *I. dammini* by means of a 6-year survey on a New England island that does not have endemic *I. dammini*. In addition, we determine whether there is *Borrelia burgdorferi* infection in these transported ticks and assess their role in the introduction of enzootic Lyme disease.

Materials and Methods

Study site. Appledore Island (33.6 hectares) is in the Isles of Shoals archipelago, 9.7 km off the Maine–New Hampshire coast

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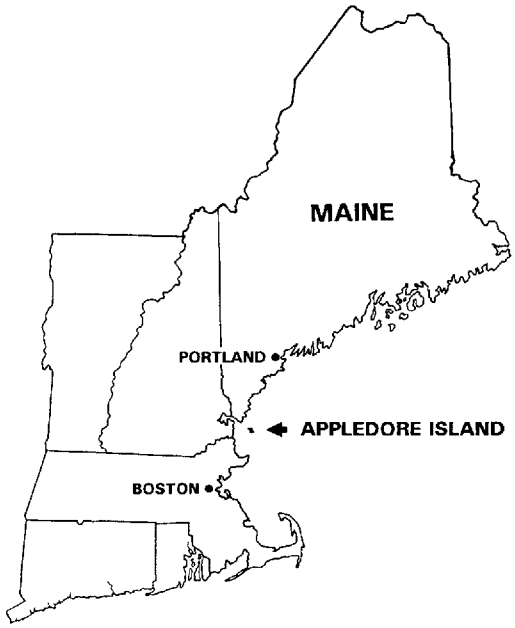

Figure 1. Map of area of study of bird migration and tick dispersal.

(figure 1). Lyme disease is endemic on a coastal site ~37 km south-southwest of Appledore Island. A dry shrub habitat covers most of the island, with species of the rose and heath families and poison ivy predominant. Small freshwater and saltwater marshes are present, as is a moist shrub alder thicket. Mammal species present include Norway rats *(Rattus norvegicus)* and muskrats *(Ondatra zibethicus),* but deer and mice are absent. *I. dammini* ticks have not successfully colonized the island, though questing nymphal and adult ticks have been collected there on occasion. There are no year-round human residents on the island, but a marine education facility is occupied each summer.

Vector surveys. Two ornithologists (D.W.H., S.R.M.) experienced in bird-banding supervised the vector survey during the spring (May through early June) and fall (August to September) migrations during the years 1989–1994 as part of an established bird-banding program. Captured birds were examined on the head and neck for ticks. All ticks were removed by forceps, placed in charcoal-based plastic vials, and transported to the Maine Medical Center Research Laboratory for identification by a scientist skilled in tick identification (E.H.L.). Recorded data included the number of ticks on each bird species, the proportion of infested birds among those captured, and the date of collection. Data were not obtained during the fall of 1992 due to inadequate field supplies.

To determine whether *I. dammini* ticks had colonized Appledore Island, rats and muskrats were captured in 40.6 × 12.7 × 12.0 cm live traps (model 102; Tomahawk Live Trap, Tomahawk, WI). Captured rats and muskrats were anesthetized with halothane and examined for ticks. An ear punch biopsy was obtained for isolation of *B. burgdorferi* as described by Sinsky et al. [6]. In addition, questing ticks were sought by flagging vegetation with 1 × 1 m flags during June (for nymphal ticks) and October (for adult ticks).

Detection and characterization of B. burgdorferi. Live *I. dammini* ticks were dissected and their midguts examined for *B. burgdorferi* by indirect fluorescence using a polyclonal rabbit immune serum with high titers against *B. burgdorferi* sensu lato (developed by S. Telford, Harvard University, Boston) [7]. After incubation for 1 h at 37°C, slides were further incubated with fluorescein isothiocyanate–labeled anti-rabbit IgG for 1 h more. Slides with ≥3 distinct spirochetal forms on examination with ×400 epifluorescence microscopy were considered positive for infection.

Tick midguts were also placed in supplemented BSK medium and incubated at 30°C as described [6]. Aliquots from inoculated tubes were examined weekly for 8 weeks by darkfield microscopy to detect *B. burgdorferi.* Ticks were considered infected if spirochetes were observed by either direct fluorescence assay (DFA) or culture.

DNA was isolated from 6 positive *B. burgdorferi* cultures using a standard phenol extraction protocol at a laboratory at the University of Maine (Orono) separate from that used for *Borrelia* cultures and tick identification and dissection. Positive and negative controls were run concurrently. The outer-surface protein B (OspB) gene was amplified by using the polymerase chain reaction (PCR).

Three primer sets directed at the OspB gene [8] were used for amplification. Reactions were done in a thermal cycler (model 480; Perkin-Elmer Cetus, Norwalk, CT). Components were denatured at 93°C for 30 s, annealed at 50°C for 1 min, and extended at 72°C for 2 min, for a total of 35 cycles. Double-stranded PCR amplification products were separated on 1% SeaPlaque-mediated agarose gels (FMC BioProducts, Rockland, ME). The bands were treated with agarase and cycle-sequenced using Taq polymerase–mediated incorporation of dye-labeled dideoxy terminators (Applied Biosystems, Foster City, CA). Samples were run through Centri-Sep columns (Princeton Separations, Adelphia, NJ) to remove unincorporated nucleotides. Purified samples were analyzed in a 373A DNA sequencer (Applied Biosystems) and aligned with New York strains B31 and 19535 using the Seq Ed 675 Q DNA sequence editor program (Applied Biosystems) on a MacIntosh Quadra 950 computer.

Results

Vector surveys. The proportion of spring and fall migrants infested with *I. dammini* is noted in table 1, and the species most commonly harboring ticks among spring migrants are listed in table 2. Of >90 bird species examined, 15 carried *I. dammini* ticks. One species, the common yellowthroat, accounted for 65% of *I. dammini* ticks. Additional tick species removed from birds included *Dermacentor variabilis* (1), *Ixodes brunneus* (2), *Ixodes muris* (3), *Amblyomma maculatum* (3), and numerous *Haemaphysalis leporispalustris.* Only *I. dammini* ticks were examined for spirochetes. No *Ixodes dentatus* ticks were found, but this species has been identified on rare occasions in our other surveys in Maine and is easily distinguished from *I. dammini.*

Fall flagging efforts (12 person-hours) resulted in the collection of 3 adult *I. dammini,* a collection rate far lower than seen on other Maine coastal islands with endemic populations of *I. dammini.* No nymphal ticks were collected from vegetation in late spring by flagging (4 person-hours). No ticks were present on 11 captured rats and 3 muskrats. Ear punch biopsies were

Table 1. *I. dammini* infestation of migratory passerine birds, Appledore Island, Maine, 1989–1994.

Year	Spring				Fall			
	Total no. of birds examined	No. (%) infested with *I. dammini*	No. of ticks by stage		Total no. of birds examined	No. (%) infested with *I. dammini*	No. of ticks by stage	
			Nymphs	Larvae			Nymphs	Larvae
1989	506	3 (0.6)	4	0	2167	2 (0.1)	0	2
1990	1445	11 (0.8)	17	2	1258	0	0	0
1991	1967	9 (0.5)	12	2	1465	0	0	0
1992	2483	60 (2.4)	154	1	—	—	—	—
1993	2396	24 (1.0)	38	1	1045	10 (1.0)	1	43
1994	2527	30 (1.2)	84	4	2672	7 (0.3)	7	13
Summary	11,324	137 (1.2)	309	10	8607	19 (0.2)	8	58

negative by culture for *B. burgdorferi* from the 2 rats and 2 muskrats tested.

Tick infection rate and characterization of B. burgdorferi. Of 267 ticks examined by polyclonal DFA, 53 were positive for spirochetes, and *B. burgdorferi* was isolated from 11 of 110 cultures from ticks. Among 108 ticks that were both cultured and examined with DFA, concordance of results was 93.5%. Table 2 summarizes tick infection rates by *B. burgdorferi* among spring migrants. Six *B. burgdorferi* isolates were characterized by OspB sequencing and found to be identical to 2 prevalent strains from other areas of the Maine coast [9].

Of note was the presence of spirochetes in 2 larval ticks. One *I. dammini* larva was removed during spring migration from a swamp sparrow *(Melospiza georgiana)* that had *I. dammini* nymphs feeding on it as well, and *B. burgdorferi* was documented by immunofluorescent antibody test and by culture. The second larval *I. dammini* was removed from a northern waterthrush *(Seiurus noveboracensis)* and represented the only larva from the fall migrants examined that was positive for *B. burgdorferi.*

Discussion

Our study documents the dispersal of *I. dammini* by migrating birds from areas where this tick is endemic to an offshore island where it is not. As passerine birds on migration fly as much as 950 km/day, and subadult *Ixodes* ticks may remain on birds for 2–4 days, long-distance dispersal of *I. dammini* can be accomplished during migration of birds from areas where the tick is endemic to distant areas where it is not. Our reliance on quick visual inspection undoubtedly underestimates actual frequencies of bird infestation by ticks and, in particular, larvae because of their small size. Nevertheless, annual data provided estimates of infestation frequencies of 0.5%–2.4% (spring) and 0–1.0% (fall). Although no canopy-feeding birds harbored ticks, substantial numbers of bird species that forage on the

Table 2. *I. dammini* infestation and infection rate with *B. burgdorferi* by bird species during spring migration.

Species	Total no. of birds examined	No. (%) infested	Infection rate with *B. burgdorferi*			
			Nymphs		Larvae	
			No. tested	No. infected (%)	No. tested	No. infected
Veery (*Catharus fuscescens*)	28	4 (14.3)	12	1 (8.3)	0	0
Gray-cheeked thrush (*C. minimus*)	7	3 (42.9)	3	0	0	0
Swainson's thrush (*C. ustulatus*)	55	3 (5.5)	20	4 (20)	1	0
Hermit thrush (*C. guttatus*)	45	3 (6.7)	5	1 (20)	0	0
Wood thrush (*Hylocichla mustelina*)	19	1 (5.3)	2	0	0	0
Ovenbird (*Seiurus aurocapillus*)	154	4 (2.6)	7	0	1	0
Northern waterthrush (*S. noveboracensis*)	252	8 (3.2)	10	4 (40)	1	0
Common yellowthroat (*Geothylpis trichas*)	2585	100 (3.9)	177	42 (23.7)	1	0
Swamp sparrow (*Melospiza georgiana*)	104	4 (3.8)	7	1 (14)	1	1

NOTE. Data are for only the 9 most commonly infested species.

ground in moist forested or shrubby habitats were infested with *I. dammini* at frequencies of 2.6%–42%. These birds provide a means of dispersal of multiple ticks between discontinuous patches of suitable habitat, increasing the likelihood of successful colonization after dispersal to a new area.

Our study site is located north of the most highly Lyme disease–endemic areas in the northeastern United States; therefore, it is not surprising that tick importation was greatest during spring migration when birds were more likely to be arriving from these areas. A survey site south of the areas with endemic Lyme disease might reveal a reversal of this pattern, with fall migration accounting for the period of maximal tick dispersal, as previously hypothesized by Spielman et al. [10].

Despite regular importation of deer ticks to Appledore Island by birds, we found no evidence of successful colonization of this island by ticks. As the habitats on Appledore Island are similar to those on nearby coastal islands that have endemic *I. dammini,* we assume that absence of deer or other large mammals on Appledore Island prohibits completion of the tick life cycle. Although mice are also absent, we have previously shown the persistence of enzootic Lyme disease on another Maine island, where Norway rats serve as the primary host for juvenile *I. dammini* and reservoir host for *B. burgdorferi* in the absence of mice [11].

Our study supports an important role for migrating birds in the dispersal of deer ticks, but the establishment of enzootic Lyme disease by this means is more problematic. Although 20% of *I. dammini* nymphs removed from birds were infected with *B. burgdorferi,* these ticks are unlikely to contribute to the establishment of the enzootic because, after molting, they feed on large mammals such as deer that are not reservoir-competent and because transovarial transmission of *B. burgdorferi* is rare. Partially fed nymphs might infect rodents or other competent reservoir hosts, but the frequency of this is unknown. Infected larval ticks, though rarely encountered in this study, would likely feed on rodents after molting to nymphs and could introduce Lyme disease by this means. Previous studies provide evidence that some bird species may be competent reservoir hosts for *B. burgdorferi,* a possible explanation of the presence of the infection in 2 larval ticks in our survey [4, 5, 12–14].

These birds, once infected, could contribute to the establishment of enzootic Lyme disease through continued infection of larval ticks that infest them. The importance of this mechanism for disease introduction is difficult to assess in the absence of data on the duration of spirochetemia in these birds.

Characterization by OspB sequencing of 6 isolates of *B. burgdorferi* obtained from infected ticks revealed the presence of 2 strains that predominate in the northeastern United States and are established in other areas of coastal Maine within 37 km of Appledore Island. No unusual strains were identified in this study, but documentation of multiple strains of *B. burgdorferi* on several Maine islands [9] supports the possibility of multiple introductions.

Our data document similar frequencies of bird infestation by *I. dammini* as those demonstrated previously in studies of the dispersal of *I. ricinus* during bird migration in Europe [15]. Awareness of the role of bird migration in the dispersal of *I. dammini* ticks contributes to our understanding of the changing epidemiology of Lyme disease and other diseases (e.g., babesiosis, ehrlichiosis) associated with this tick vector.

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