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First Isolation of Lyme Disease Spirochete, *Borrelia burgdorferi*, from Ticks Collected from Songbirds in Ontario, Canada

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ABSTRACT

This study documents the first isolation of the Lyme disease spirochete, Borrelia burgdorferi, from ticks attached to songbirds in Ontario. Viable spirochetes were cultured from a nymph of the blacklegged tick, Ixodes scapularis Say, detached from a Hermit Thrush, Catharus guttatus. Similarly, B. burgdorferi isolates were obtained from I. scapularis nymphs collected from a House Wren, Troglodytes aedon. These B. burgdorferi isolates show divergent heterogeneity indicating that some strains originated from nearby locales and others from southern latitudes. Lyme disease vector ticks are transported by northward-migrating songbirds, and dispersed widely across southern Canada. Importantly, B. burgdorferi-infected I. scapularis ticks pose a public health risk to people and domestic animals wherever they are released.

INTRODUCTION

The blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodidae), the primary vector of the spirochete that causes Lyme disease, is distributed widely east of the Rocky Mountains by migratory songbirds (Anderson et al. 1990, Durden and Keirans 1996). Typically, *I. scapularis* immature stages (larvae, nymphs) parasitize ground- and shrub-frequenting passerine species. En route to breeding grounds, northward-migrating passerines make landfall at established populations of *I. scapularis*, which are scattered across northern United States (Steiner et al. 2008) and southern Canada, and some of these ticks are infected with the Lyme disease spirochete, *Borrelia burgdorferi* (Scott et al. 2001, Morshed et al. 2005, Ogden et al.

2008). At these stopovers, ground-foraging songbirds meander through grassy vegetation and arboreal leaf litter and are parasitized by bird-associated ticks. Fully engorged ticks later detach from these migrating songbirds farther afield in cool, humid, tick-friendly habitats where *I. scapularis* immature stages subsequently molt in 5-11 weeks to the next life stage. From mid-May to early June, northward migration of passerines overlaps directly with the peak questing activity of *I. scapularis* nymphs. Ogden et al. (2008) estimated 50 million to 175 million *I. scapularis* ticks are dispersed by birds annually across Canada.

Anderson and Magnarelli (1984) pioneered bird-tick-*Borrelia* studies in the Lyme, Connecticut, area and cultured *B. burgdorferi* from the liver of a Veery, *Catharus fuscescens*, and *I. scapularis* larvae attached to it. In Canada, Scott et al. (2001) initially cultured *B. burgdorferi* from a live, fully engorged *I. scapularis* nymph, which was collected in May 1999 from a Common Yellowthroat, *Geothlypis trichas*, in Nova Scotia. Subsequently, *B. burgdorferi* was detected in Ontario in an ethanol-preserved *I. scapularis* nymph collected in May 2003 from a Common Yellowthroat, which was mist-netted at Long Point, Ontario (Morshed et al. 2005). More recently, *B. burgdorferi* has been detected in *I. scapularis* immature stages collected from songbirds in Ontario (Ogden et al. 2008, Scott et al., unpubl. data) because these ticks were dead specimens, spirochetes were mortal.

The aim of this study was to culture live *B. burgdorferi* from subadult *I. scapularis* collected from songbirds in Ontario, and to determine the

viability and genetic variability of Lyme disease spirochetes in these bird-feeding ticks.

METHODS

Bird banders captured songbirds with mist nets, and ticks were removed using stainless steel tweezers (superfine tip). Ticks were inserted into self-sealing micro tubes, which contained slightly moistened, non-chlorinated filter paper. These ticks were sent by courier to Scott for identification, and ticks indigenous to southern latitudes were forwarded to Durden for confirmation of identification. In the molecular laboratory, individual live ticks were cultured in BSK-H media, and cultures were checked weekly by dark-field microscopy for four weeks.

PCR amplification was performed on the ribosomal *rrf* (5S)-*rml* (23S) intergenic spacer gene and the linear plasmid *OspA* gene using the Stratagene Robocycler as described previously (Morshed et al. 2005). The *rrf-rml* amplicons were sequenced with the ABI 3130 DNA sequencer and analyzed with DNASTar software.

The St. Williams Crown Forest reference isolate ON05FTP863 (GenBank accession no. FJ707480) cultured from an *I. scapularis* female is accessible via the GenBank website:
<http://www.ncbi.nlm.nih.gov/nuccore/224981432>.

RESULTS

A partially engorged *I. scapularis* nymph (Fig. 1) was removed from a Hermit Thrush, *Catharus guttatus*, which was mist-netted at Long Point, Ontario (42.51N, 80.16W) on 13 May 2007. Likewise, six engorged *I. scapularis* subadults (five sent for identification) were collected from a House Wren, *Troglodytes aedon* on 14 May 2007. Motile spirochetes were observed by dark-field microscopy in cultures from a single nymph from the Hermit Thrush and two nymphs from the House Wren.

PCR amplification of the *rrf-rml* intergenic spacer and *OspA* genes confirmed that the three isolates belong to the *B. burgdorferi sensu lato* complex. None of the three isolates had the same nucleotide sequences in the *rrf-rml* region; they differed from



Fig. 1. Motile life stages of blacklegged tick, *Ixodes scapularis*: unfed larva, unfed nymph, male, unfed female. Bar, 3 mm.

Photo by John D. Scott

Table 1. Isolates of *Borrelia burgdorferi* cultured from *I. scapularis* ticks collected from songbirds at Long Point, Ontario

Bird species	Tick stage, tick no.	Isolate	Amplicon size (bp) ^b	Genbank accession no.	Deviation ^c from strain B31	Website reference ^a http://www.ncbi.nlm.nih.gov/nuccore/224981433
Hermit Thrush	nymph, 075A23	ON07FTP979	220	FJ707481	57	gov/nuccore/224981433
House Wren	nymph, 07-5A24C	ON07FTP981	218	FJ707482	3	gov/nuccore/224981434
	nymph, 07-5A24D	ON07FTP982	218	FJ707483	2	gov/nuccore/224981435

^a The website prefix is provided in the subheading and the suffixes in the column below.

^b bp, number of nucleotides sequenced in the *rrf* (5S)-*rrl* (23S) intergenic spacer amplicon.

^c deviation within *rrf-rrl* region, in bp, from type strain B31.

each other by at least 3 base pairs (bp). The two isolates (ON07FTP981 [nymph 07-5A24C] and ON07FTP982 [nymph 07-5A24D]), which were collected from the House Wren (Table 1), had nucleotide sequence differences from the *B. burgdorferi sensu stricto* type strain B31 consisting of 3 bp and 2 bp, respectively. In contrast, the isolate ON07FTP979 from the nymph (07-5A23), which was collected from the Hermit Thrush, had a 2-bp insertion plus 57-bp substitutions in the *rrf-rrl* region, compared to strain B31.

DISCUSSION

The present study shows that migratory songbirds disperse *I. scapularis* ticks in Ontario harboring *B. burgdorferi* with diverse heterogeneity. The *B. burgdorferi* isolate in the nymph attached to the Hermit Thrush, which winters as far south as Guatemala, had a marked difference (2-bp insertion, 57-bp substitutions) in the *rrf-rrl* 220-bp region. Researchers (Lin et al. 2004, Rudenko et al. 2009) report genetic diversity of *B. burgdorferi* in ticks and vertebrate hosts collected in the southern United States and, ultimately, these bird-associated ticks can be transported to Canada during spring migration. Because of the large number of nucleotide substitutions, it is likely that the isolate, which was cultured from the nymph attached to the Hermit Thrush, originated from southern United States.

The isolates from the two *I. scapularis* nymphs collected from the House Wren deviated from each other by three nucleotides in the *rrf-rrl* 218-bp amplicon. Even though certain songbirds, such as the American Robin, *Turdus migratorius*, may be competent reservoirs of *B. burgdorferi* (Richter et al. 2000), endogenous transmission of *B. burgdorferi* in our study via the House Wren was not evident during tick engorgement. One isolate (ON07FTP981) matched a Turkey Point Provincial Park isolate (ON06FTP933; Scott et al. 2008), and also it matched an isolate (ON05FTP863) cultured from an *I. scapularis* female collected at St. Williams Crown Forest at Turkey Point, Ontario. These isolates have been characterized as *B. burgdorferi sensu stricto* (Scott et al. 2008); this genospecies is common across North America, and causes Lyme disease in people and domestic animals. Genographically, the combination of these Lyme disease spirochetes reveals a direct genetic link between Turkey Point Provincial Park, St. Williams Crown Forest, and Long Point. The other isolate (ON07FTP982) cultured from the nymph on the House Wren had a 2-bp deviation from strain B31. In essence, at least one of the *B. burgdorferi* isolates was acquired by the House Wren in the Long Point region.

Bird-tick studies reveal evidence of *B. burgdorferi*-positive *I. scapularis* introduced by spring migrants across northern latitudes of central and eastern Canada. Ogden et al. (2008) reported that

approximately 15% of the *I. scapularis* nymphs, which were collected from infested songbirds in eastern Canada, were positive for *B. burgdorferi*. During a 10-year tick-host study, Morshed et al. (2006) revealed an infection prevalence of 12.9% for *B. burgdorferi* in *I. scapularis* adults, which were collected from dogs, cats, horses, and humans as far north as the 50° N in Ontario. Similarly, Ogden et al. (2006) reported 12.5% of the *I. scapularis* (adults) collected from people, domestic and wildlife animals from Manitoba to Newfoundland and Labrador, to be infected with *B. burgdorferi*. The close correlation between bird-derived immature stages and mammalian-derived *I. scapularis* adults accentuates the fact that migratory passerines are dispersing agents of *B. burgdorferi*-infected blacklegged ticks in Ontario.

A heavily parasitized songbird, which drops ticks in one site, can start a new population of *I. scapularis*. Anderson and Magnarelli (1984) reported high infestations of *I. scapularis* (reported as *I. dammini*) on passerines in Connecticut: 21 *I. scapularis* larvae were removed from both a Gray Catbird, *Dumetella carolinensis* and a Swamp Sparrow, *Melospiza georgina* and, likewise, 19 *I. scapularis* nymphs from an American Robin. In the present study, six *I. scapularis* subadults were collected from the House Wren and, ultimately, provide the potential for *I. scapularis* and *B. burgdorferi* to become established in a new locale.

Recently, Stutchbury et al. (2009) used light-weight geolocators on passerines to track migratory routes, and found that certain songbirds fly 577 km/d or more during spring migration and, during a typical five-day engorgement period, can import bird-feeding *I. scapularis* nymphs from the southern United States into Canada. Aided by south winds and warm temperatures, southern temperate and Neotropical songbirds, including House Wrens and Hermit Thrushes, are able to import ticks into Ontario with a multitude of *B. burgdorferi* strains and other tick-associated pathogens.

Not only does the present study show viability of *B. burgdorferi* in bird-derived *I. scapularis*, it also reveals diverse heterogeneity of *B. burgdorferi*-

infected ticks imported by migratory songbirds. Consistent with other studies (Morshed et al. 2006, Rudenko et al. 2009), our study shows that *B. burgdorferi* strains in Ontario are more heterogeneous than previously thought. Ultimately, this genetic diversity could substantially alter clinical manifestations and serological responses in Lyme disease patients and, as a result, may lead to a misdiagnosis. Ornithologists and medical practitioners need to be aware that outdoors people may encounter Lyme disease vector ticks that can result in diverse pathological symptoms.

In conclusion, songbirds act as hosts for bird-transported ticks and play an integral role in the enzootic cycle of Lyme disease. Not only do passerines act as transporting agents of *I. scapularis* harboring viable *B. burgdorferi*; but they may also act as reservoir-competent hosts. The heightened questing activity of *I. scapularis* nymphs in May and early June and the concurrent tick parasitism of passerine migrants during peak spring migration ostensibly show that these avian hosts are long-range dispersing agents of Lyme disease vector ticks. Because migratory songbirds transport *B. burgdorferi*-infected *I. scapularis* anywhere in Ontario, people do not have to visit an endemic area to contract Lyme disease.

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