

First Record of *Ixodes affinis* Tick (Acari: Ixodidae) Infected with *Borrelia burgdorferi* Sensu Lato Collected from a Migratory Songbird in Canada

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Received date: April 14, 2016; Accepted date: June 25, 2016; Published date: June 30, 2016

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Abstract

Migratory songbirds transport hard-bodied ticks (Acari: Ixodidae) into Canada during northward spring migration, and some of these bird-feeding ticks harbor a wide diversity of pathogenic microorganisms. In this study, we collected a nymphal *Ixodes affinis* Neumann from a Common Yellowthroat, *Geothlypis trichas* (Linnaeus), at Ste-Anne-de-Bellevue, Québec, and it was infected with the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt & Brenner. Using PCR on this tick extract and DNA sequencing on the borrelial amplicons, we detected *B. burgdorferi* sensu stricto (s.s.), a genospecies that is pathogenic to people and certain domestic animals. In addition, we collected an *I. affinis* nymph from a Swainson's Thrush, *Catharus ustulatus* (Nuttall), at Toronto, Ontario, and a co-feeding nymphal blacklegged tick, *Ixodes scapularis* Say, tested positive for *B. burgdorferi* s.s. These bird-tick findings constitute the first reports of *I. affinis* in Ontario and Québec and, simultaneously, the first report of a *B. burgdorferi* s.l.-infected *I. affinis* in Canada. Since Neotropical and southern temperate songbirds have a rapid flight pace, they are capable of transporting ticks infected with *B. burgdorferi* s.l. hundreds of kilometres to Canada. Health-care professionals should be cognizant that migratory songbirds can transport diverse genotypes of *B. burgdorferi* s.l. into Canada from southern latitudes that may be missed by current Lyme disease serological tests.

Keywords: Tick; *Ixodes affinis*; *Borrelia burgdorferi*; Lyme disease; Migratory songbirds; Bird parasitism; Eastern Canada

Abbreviations:

PCR: Polymerase Chain Reaction

Introduction

Ixodes affinis Neumann (Acari: Ixodida: Ixodidae) is an obligate, blood-sucking ectoparasite that feeds on mammals and birds [1,2]. Biogeographically, this ixodid (hard-bodied) tick is native to Central and South America [3], and occurs in the United States from Florida to Virginia [2,4,5]. In the coastal plains of the Mid-Atlantic and southeastern United States, *I. affinis* parasitizes a wide

array of mammals, including cotton mouse, *Peromyscus gossypinus* (LeConte); cotton rat, *Sigmodon hispidus* Say and Ord; marsh rice rat, *Oryzomys palustris* Harlan; southern short-tailed shrew, *Blarina carolinensis* (Bachman); gray squirrel, *Sciurus carolinensis* Gmelin; eastern cottontail, *Sylvilagus floridanus* (J.A. Allen); raccoon, *Procyon lotor* (Linnaeus); *Virginia opossum*, *Didelphis virginiana* (Kerr); domestic cat, *Felis catus* (L.); dog, *Canis lupus familiaris* L.; bobcat, *Lynx rufus* (Schreber); cougar, *Puma concolor* (L.); white-tailed deer, *Odocoileus virginianus* Zimmermann; and American black bear, *Ursus americanus* (Pallus) [2]. In particular, the cotton mouse and the cotton rat are major reservoir hosts for *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt & Brenner in the southeastern United States [6]. Of biological and medical significance,

I. affinis enhances the epidemiological cycle of *B. burgdorferi* s.l., especially within indigenous areas [7].

Songbirds (Passeriformes) are avian hosts of *I. affinis* and *I. scapularis* larvae and nymphs [1,5,8]. While en route to their breeding grounds, passerine migrants make landfall at Lyme disease endemic areas, and are often parasitized by host-seeking ticks. Since peak questing activity of *I. scapularis* and *I. affinis* nymphs coincides with peak northward songbird migration, ground-frequenting passerines are often parasitized by both of these nymphal species. In Canada, Scott et al. [8] reported *I. affinis* and *I. scapularis* on a Swainson's Thrush, *Catharus ustulatus* (Nuttall), in south-central Manitoba during northward spring migration. In a recent study, Heller et al. [5] reported *I. affinis* immatures on 6 different passerine species in southeastern Virginia, and one songbird-derived nymph was infected with *B. burgdorferi* s.l.

Based on tick-host-pathogen studies in the southeastern United States, researchers found that *I. affinis* is a competent vector of the Lyme disease bacterium, *B. burgdorferi* s.l. [6,7]. Globally, the *B. burgdorferi* s.l. complex consists of at least 23 genospecies or genomospecies. In North America, at least nine *B. burgdorferi* s.l. genospecies are present, namely *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s.), *B. californiensis*, *B. carolinensis*, *B. garinii*, *B. kurtenbachii*, and *B. mayonii* [9-16]. Of these genospecies, *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* s.s., *B. garinii*, and *B. mayonii* are known to be pathogenic to humans [16-20].

The aim of the present study was to expound on the movement of songbird-transported ticks in the Northern Hemisphere during northward spring migration, and report any novel tick-host associations in Canada. In addition, we wanted to determine whether any ticks on Neotropical and southern temperate songbirds are infected with *B. burgdorferi* s.l.

Materials and Methods

Tick collection

During spring migration, *I. affinis* and *I. scapularis* nymphs were collected from songbirds by bird banders at four different sites in eastern Canada. Nymphs were removed using fine-pointed, stainless steel forceps, and stored in polypropylene vials that were labelled with background information (i.e., bird species, collection date, geographic location, collector's name). The vented vials were placed in a self-sealing, double-zipped plastic bag

with slightly moistened paper towel. The ticks were promptly sent by express mail to the tick identification laboratory (J.D.S.). The nymphal ticks were tentatively identified using a taxonomic key [21]. After fully engorged nymphs moulted to adults, they were put in 94% ethyl alcohol, and sent by overnight courier for confirmation of identification (L.A.D.).

Spirochete detection

In phase 1, ticks were sent to a separate laboratory (K.L.C.) for *B. burgdorferi* s.l. testing and molecular analysis. DNA extraction, PCR testing, and DNA sequencing were performed as previously described [22].

In phase 2, four *I. affinis* nymphs, which were collected in 2016, were sent to another laboratory (J.E.F) for PCR testing and molecular analysis. The flagellin gene was used on these *I. affinis* ticks. The primers include:

External:
FlaLL: ACATATTCAGATGCAGACAGAGGT;
FlaRL: GCAATCATAGCCATTGCAGATTGT;

Internal:
FlaLS: AACAGCTGAAGAGCTTGGGAATG;
FlaRS: CTTTGATCACTTATCATTCTAATAGC.

The methodology for DNA extraction, PCR testing, and DNA sequencing is outlined in the scientific articles [23-26].

Nucleotide sequence accession numbers

The DNA sequence of the 194-bp (base position 313 to 506), and the 206-bp (base position 532 to 737) amplicons of *B. burgdorferi* s.l. flagellin (*flaB*) gene, which were acquired from the *I. affinis* nymph (15-5A51) using PCR1 and PCR2 primer sets have been deposited in the GenBank data base with accession number: KX011447. In addition, *B. burgdorferi* s.l. amplicons from the *I. scapularis* nymph (15-5A47A) using PCR1 and PCR2 primer sets have been submitted to the GenBank database with accession number: KX011446.

Results

Six *Ixodes* nymphs were collected from five species of passerine birds at four different collection sites in eastern Canada (Figure 1). The vector-host-pathogen associations are listed in Table 1. A partially engorged *I. scapularis* nymph (15-5A47A) and a fully engorged *I. affinis* nymph (15-5A47B) were collected from a Swainson's Thrush on 24 May 2015 at Toronto, Ontario.

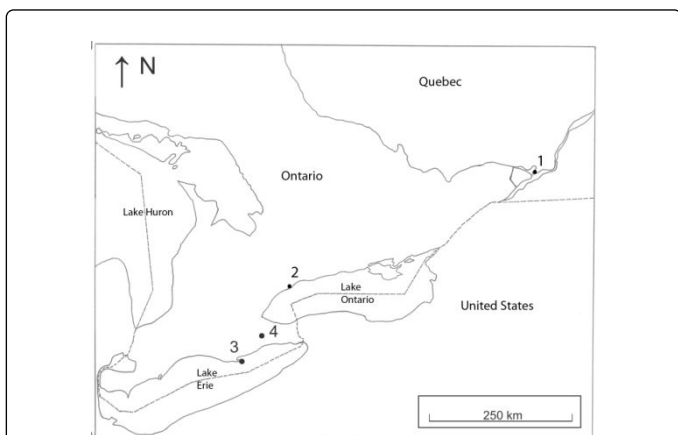


Figure 1: Geographic locations in eastern Canada where ticks (*I. affinis*, *I. scapularis*) were collected from migratory songbirds: 1. McGill Bird Observatory, Ste-Anne-de-Bellevue, Québec, 45.43°N, 73.94°W; 2. Tommy Thompson Park Bird Research Station, Toronto, Ontario, 43.63°N, 79.33°W; 3. Long Point Bird Observatory, Tip of Long Point, Ontario, Canada, 42.55°N, 80.05°W; and 4. Ruthven Park National Historic Site Banding Station, Haldimand Bird Observatory, Cayuga (York), Ontario, 42.97°N, 79.87°W. Parenthesis indicates mailing address.

The *I. scapularis* nymph moulted to a female in 39 d, whereas the *I. affinis* nymph moulted to a female in 43 d. The *I. scapularis* nymph tested positive for *B. burgdorferi* s.l., and the *I. affinis* was negative. This bird parasitism is the first record of *I. affinis* in Ontario and, simultaneously, the first documentation of *I. affinis* on a bird in this province.

At Ste-Anne-de-Belleville, Québec, a partially engorged *I. affinis* nymph (15-5A51) was collected from a Common Yellowthroat, *Geothlypis trichas* (Linnaeus), a southern temperate songbird, on 26 May 2015 (Figure 2). The nymph tested positive for *B. burgdorferi* s.l. and, based on DNA sequencing, was validated as *B. burgdorferi* s.s. The discovery of *I. affinis* nymphs in Québec constitutes a new extralimital distribution record for this tick species. As well, it is the first account of *I. affinis* on birds in Québec and, notably, the first report of a *B. burgdorferi* s.l.-infected *I. affinis* in Canada.



Figure 2: Common Yellowthroat, male, parasitized by a partially engorged *Ixodes* sp. nymph. Arrow points to engorged nymph. Photo credit: late Bronwyn Dalziel.

Upon DNA sequencing and molecular analysis, the three *B. burgdorferi* s.l. amplicons were determined to be *B. burgdorferi* s.s., a genospecies pathogenic to humans and certain domestic animals.

Discussion

We document the first records of *I. affinis* in eastern Canada, and the first *B. burgdorferi* s.l.-infected *I. affinis* in Canada. Our vector-host-pathogen findings show that Neotropical and southern temperate songbirds transport *I. affinis* into Canada during northern spring migration and the southern United States. Not only are migratory songbirds transporting ixodid ticks into Canada, they are importing *B. burgdorferi* s.l.-infected ticks.

Bird species	Site*	Date Collected	Life Stage	PCR	Geno-species
Ontario					
Swainson's Thrush <i>Catharus ustulatus</i> (Nuttall)	2	24 May 2015	N†	neg.	—
Slate-colored Junco <i>Junco hyemalis</i> (Linnaeus)	3	17 Apr 2016	N	neg.	—
House Wren <i>Troglodytes aedon</i> (Vieillot)	4	28 Apr 2016	N	neg.	—
Swainson's Thrush <i>Catharus ustulatus</i> (Nuttall)	2	21 May 2016	N	neg.	—
Québec					
Common Yellowthroat <i>Geothlypis trichas</i> (L.)	1	26 May 2016	N	pos.	Bbss
Northern Waterthrush <i>Parkesia noveboracensis</i> (Gmelin)	1	10 May 2016	N	neg.	—

*See Figure 1 for locations.

†The host bird was co-infested with an *I. scapularis* nymph; N, nymph Bbsl., *B. burgdorferi* sensu lato; Bbss, *B. burgdorferi* sensu stricto

Table 1: Detection of *B. burgdorferi* s.l. in *Ixodes affinis* collected from passerine birds in eastern Canada, by province, 2015-2016.

***Ixodes affinis* transported to Canada**

In this study, a Common Yellowthroat, a southern temperate songbird, transported a *B. burgdorferi* s.s.-infected *I. affinis* nymph to Ste-Anne-de-Bellevue, Québec. At repletion, this fully engorged nymph could have moulted to a female in the wild and, subsequently, could have attached to a dog or cat or another suitable wild mammal, and transmitted Lyme disease spirochetes to the host. In a separate bird-tick association, a Swainson's Thrush was parasitized by an *I. affinis* nymph that was co-feeding with the *B. burgdorferi* s.s.-infected *I. scapularis* nymph. If the Swainson's Thrush became spirochetemic during co-infestation, the *I. affinis* nymph could also have become infected with *B. burgdorferi* s.s. by the time of repletion. During the co-feeding process, the *I. scapularis* and the *I. affinis* nymphs imbibe, regurgitate, and share the host's blood and, thus, the *I. affinis* nymph could have subsequently acquired Lyme disease spirochetes. After the *I. scapularis* nymph and the *I. affinis* nymph dropped from the Swainson's Thrush, they would have instinctively descended into the cool, moist leaf litter, and later moulted to adults and, ultimately, parasitized vertebrate hosts, including humans.

Anderson and Magnarelli [27] cultured *B. burgdorferi* s.l. from a Common Yellowthroat, and revealed that this bird species is a reservoir-competent host. As well, tick researchers have collected *B. burgdorferi* s.l.-infected *I. scapularis* larvae from Common Yellowthroat which reinforces host competency of this bird species [27-29]. Since transovarial transmission of *B. burgdorferi* s.l. does not typically occur in *I. scapularis* [30], this tick species must acquire *B. burgdorferi* s.l. during a blood meal. Either the *I. affinis* nymph acquired *B. burgdorferi* s.s. directly from the Common Yellowthroat or, as a larva, acquired the infection while feeding on a spirochetemic host.

Importation of *B. burgdorferi* s.l. into Canada

Migratory songbirds are a natural mode of transporting *B. burgdorferi* s.l.-infected ticks into Canada. In this study, passerine migrants transported *B. burgdorferi* s.s.-infected *I. affinis* and *I. scapularis* immatures into eastern Canada. Not only have *B. bissettii* and *B. burgdorferi* s.s. been isolated from both of these *Ixodes* species in the southeastern United States, *B. carolinensis* was detected in a Carolina Wren, *Thryothorus ludovicianus* Vieillot [31], in the same region. Consequently, it would certainly be plausible for a passerine migrant to transport *I. affinis* immatures into Canada infected with *B. bissettii*, *B. burgdorferi* s.s., and *B. carolinensis*.

In Canada, Banerjee et al. [32] cultured *B. burgdorferi* s.l. (isolate BC93T1340) from *Ixodes angustus* Neumann detached from a wild deer mouse, *Peromyscus maniculatus* Gloger, collected at Squamish, British Columbia; this novel borrelial isolate was later delineated as *B. bissettii* (GenBank no. AY077830). Rudenko et al. [19] provided the first successful cultivation of live *B. bissettii* from a North American patient, and the first recovery of *B. burgdorferi* s.s. from residents in the southeastern United States. Notably, these symptomatic patients were seronegative for Lyme disease, and had undergone antibiotic treatment based on the suspicion of having Lyme disease. These findings could likewise explain why some Canadian patients are seronegative for Lyme disease. Biomedical researchers have found that *B. bissettii* can be missed by current Lyme disease serology and, because of this fact, members of the health-care profession have not recognized the presence of *B. bissettii* in patients in Canada. Commercial serological tests, which are based on immune response, yield unsatisfactory results for Lyme disease screening [33], especially in the advanced disease stage, and have a sensitivity of only 46-53% in patients who have gone beyond the early disseminated stage [34]. Our study underpins the fact that passerine migrants are transporting *I. affinis* ticks into Canada from southern latitudes that are infected with *B. burgdorferi* s.s. and, potentially, harbor *B. bissettii* and *B. carolinensis* that may be missed by current serological testing.

B. burgdorferi* s.l. in patients associated with *I. affinis

The isolation of motile *B. bissettii*-like strains from patients provides evidence that diverse *Borrelia*, other than *B. burgdorferi* s.s., could cause Lyme disease in humans across North America. Globally, *B. bissettii* has been isolated from patients in California and Europe [17,35,36] and, recently, obtained from local residents in the southeastern United States [20]. Because *B. bissettii*-like strains are present in rodents, birds, and *Ixodes* ticks (i.e., *I. affinis*, *I. minor*) in the southeastern United States, their presence signals the fact that this *B. burgdorferi* s.l. genospecies is circulating in a zoonotic disease cycle between wildlife hosts and people [7].

Since *I. affinis* and *I. minor* are transported into Canada by southern temperate and Neotropical songbirds during spring migration [8,37], Canadians will likely be subject to Lyme disease-causing *B. bissettii*, *B. burgdorferi* s.s., and *B. carolinensis* from the southeastern United States and, potentially, from Central and South America.

Epidemiological cycle of *B. burgdorferi* s.l. in Canada

In the southeastern United States, *I. affinis* plays a key role in perpetuating the epidemiological cycle of *B. burgdorferi* s.l. in wildlife, and *I. scapularis* acts as a bridge vector to humans [6,7]. Such avian and mammalian parasitisms could also occur in central and eastern Canada with suitable hosts. An analogous example is seen in far-western Canada where *Ixodes spinipalpis* Hadwen & Nuttall is an enzootic vector of *B. burgdorferi* s.l., and *Ixodes pacificus* Cooley and Kohls acts as a bridge vector to humans [38]. When *I. pacificus* ticks feed on reservoir hosts, they may subsequently bite and transmit Lyme disease spirochetes to humans and other vertebrates. When *I. affinis* immatures are transported into Canada by passerine migrants, they encounter several new hosts; however, there are still many vertebrate species that are the same as those inhabiting southeastern U.S.A. Some of these similar mammalian hosts include: raccoons, Virginia opossum, white-tailed deer, American black bear, plus cats and dogs. In essence, *I. affinis* can potentially transmit *B. burgdorferi* s.l. to several reservoir hosts in Canada. Whenever any of these *B. burgdorferi* s.l.-infected hosts are bitten by *I. scapularis* immatures, they can subsequently transmit Lyme spirochetes to humans. Since migratory songbirds widely disperse Lyme vector ticks, people do not have to visit an endemic area to contract Lyme disease.

Flight pace of migratory songbirds

Many Neotropical and southern temperate songbirds have an extraordinary capability to fly long distances in a short period of time. Ogden et al. [39] conjectured that the maximum distance that songbirds can travel during a 5-day engorgement period is 425 km. However, the present study shows that the fully engorged *B. burgdorferi* s.s.-infected *I. affinis* nymph, which detached from the Common Yellowthroat at Ste-Anne-de-Bellevue, Québec, was transported much further than 425 km from its home range in the southeastern United States (Portsmouth, Virginia to Miami, Florida); the flight distance is estimated to be 670 km to 1700 km. Similarly, Scott et al. [8] reported four *I. affinis* immatures (1 nymph, 3 larvae) on a Swainson's Thrush, which is a Neotropical migrant, collected in south-central Manitoba; the estimated flight distance from the home range of these *I. affinis* specimens was approximately 1720 km (Charleston, South Carolina to Delta Marsh, Manitoba). Since the Swainson's Thrush also has a winter range from southern Mexico to South America, this passerine may have also transported *I. affinis* nymphs several thousand kilometres from the Neotropics. Stutchbury et al. [40] used light-sensitive

geolocators (nano-tags) to follow the daily flight of Neotropical songbirds, and discovered that certain passerine migrants have a flight pace of 750 km/d, or more, during northward spring migration. In addition, DeLuca et al. [41] tracked Blackpoll Warblers, *Setophaga striata* (Forster), during fall migration, and discovered they flew an average distance of 2540 km (923 km/d) during a 62-h, non-stop flight over the Atlantic Ocean. Clearly, some passerine migrants, such as the Common Yellowthroat and Swainson's Thrush, have the capacity to fly long distances much greater than 425 km during a 5-day engorgement period. The presence of Neotropical *Amblyomma* and *Ixodes* ticks on passerine migrants in Canada during spring migration provides irrefutable evidence that birds transport ticks thousands of kilometres, and disperse them widely across southern Canada [8,29,37,42-49]. When certain passerine migrants have warm temperatures, high energy diets, and southern winds, they can fly to Canada more than 10 times the distance that some researchers claim.

Recognition of *I. affinis* in northern latitudes

Even though *I. affinis* is not indigenous in Canada, this bird-transported tick is able to initiate *B. burgdorferi* s.l. infections in Canada. After the moult, this tick species can infect suitable hosts, and start a Lyme disease focus. Not only have *I. affinis* immatures been transported to Canada, other *Ixodes* species (i.e., *I. minor*) have been transported great distances from more southern latitudes [37]. Because *I. affinis* is morphologically similar to *I. scapularis*, it is likely that *I. affinis* has been misidentified as *I. scapularis*, especially in northern latitudes. Since *I. affinis* is not indigenous to Canada, the presence of larval and nymphal *I. affinis* on Neotropical and southern temperate songbirds during northbound spring migration provides confirmatory evidence that avifauna are importing *I. affinis* into Canada.

Gene mixing and diversity of Lyme disease symptoms

As an enzootic vector, *I. affinis* can directly transmit heterogeneous variants of *B. burgdorferi* s.l. to dogs, cats, and certain wildlife animals. Since *I. affinis* is transported by migratory songbirds, various strains of *B. burgdorferi* s.l. can occur within an avian host and, subsequently, be transmitted to songbird-transported ticks to northern latitudes. Rudenko et al. [31] detected *B. carolinensis* in a Carolina Wren and, likewise, in a bird-feeding *I. minor*. Of note, Scott and Durden [37] discovered an *I. minor* on a Common Yellowthroat in Canada during spring migration. Furthermore, Scott et al. [8] collected *B. burgdorferi* s.l.-positive *I. dentatus* larvae in Canada that

were collected from a Swainson's Thrush during northward spring migration. In North America, larval and nymphal *I. scapularis* have been collected from at least 78 bird species [45], and *I. scapularis* is known to harbor *B. burgdorferi* s.s. and *B. bissettii* [6]. Any combination of four passerine-transported *Ixodes* species (i.e., *I. affinis*, *I. dentatus*, *I. minor*, *I. scapularis*), which are all enzootic vectors, could be directly involved in borrelial gene mixing of *B. burgdorferi* s.l. during a long-distance migratory host flight.

The presence of 2 or more ticks, which are infected with different *B. burgdorferi* s.l. genotypes on a passerine proliferates a platform for gene mixing and microbial diversity. In particular, Scott and Durden [50] reported several *B. burgdorferi* s.l. genotypes in songbird-derived ticks collected in the Long Point bioregion. Such borrelial variants can be missed by current Lyme disease serology. In fact, Rudenko et al. [19] isolated *B. burgdorferi* s.s. and *B. bissettii*-like spirochetes from symptomatic patients, which were previously treated with antibiotics, but were seronegative for Lyme disease. The isolation of a live *B. bissettii*-like strain from a patient provides substantive evidence that this *Borrelia* genotype, and other *Borrelia* genospecies causes Lyme disease in North America [19]. Based on previous serological screening worldwide, it stands to reason that gene mixing of *B. burgdorferi* s.l. could ultimately result in inaccurate or false negative Lyme disease serology [19,33]. On the clinical front, gene mixing in songbird-transported ticks and their avian hosts could also explain the variation of Lyme disease symptomatology in dogs, cats, and humans [18].

Zoonotic and human implications

The importation of *I. affinis* on migratory songbirds reveals a unique interconnecting link between *B. burgdorferi* s.l., people, birds, ticks, domestic and wildlife hosts. *B. burgdorferi* s.s. causes a myriad of clinical manifestations in people of all ages, including profound fatigue, memory loss, inflammation, spinal or radicular pain, poor balance, sleep disturbance, head pressure, and cognitive impairment, especially in the advanced stage [51-53]. Chronic neurological Lyme disease patients often encounter tingling, numbness, burning sensations of the extremities [53]. *B. burgdorferi* s.l. lipoproteins, namely outer surface proteins and flagellin, are inducers of inflammatory cytokines. In vivo, *B. burgdorferi* s.l. has several pleomorphic forms (i.e., spirochetes, blebs, granules, round bodies) [54] and, collectively, these aggregates form gelatinous biofilm colonies [55,56]. Since *B. burgdorferi* s.l. has diverse forms, it may be recalcitrant

to treatment, especially in the advanced tertiary stage. If left untreated or inadequately treated, *B. burgdorferi* s.l. can persist in deep-seated tissues, including bone [57,58], brain [59-61], eye [62], glial and neuronal cells [63,64], ligaments and tendons [65,66], muscles [67], and fibroblasts/scar tissue [68]. Because *B. burgdorferi* s.l. can outwit the immune system by evading the innate and adaptive immune responses of the body, it can hide in immune privileged sites [69]. Several researchers have documented persistence of *B. burgdorferi* s.l. in numerous mammalian hosts, including humans [18,70-73]. In addition, Lyme disease spirochetes spread to the central nervous system, and can persist in the body for years [74-77]. Pain can be so debilitating that some patients lose their ability to function in a meaningful and productive way. Often, patients are confined to their residence, and end up on disability, and are unable to work or attend school. As *B. burgdorferi* s.l. advances and prevails in tissues and organs, this spirochetosis can result in fatal outcomes [59,76]. Whenever *B. burgdorferi* s.s. and *B. bissettii* are isolated from seronegative patients, these cultures provide irrefutable evidence that these genospecies do, in fact, cause Lyme disease. Moreover, even though infected rhesus macaques had been treated with antibiotics, Embers et al. [70] have documented *B. burgdorferi* s.l. in these seronegative primates, and conclusively determined that Lyme disease can be persistent.

In conclusion, we provide compelling proof that migratory songbirds transport *I. affinis* into Canada during northward spring migration. We document the first record of a *B. burgdorferi* s.s.-positive *I. affinis* in Canada and, synchronously, provide new host and distribution records for this tick species. Furthermore, we provide new evidence that *I. affinis* can be added to the list of competent enzootic vectors of *B. burgdorferi* s.l. in Canada. After *I. affinis* immatures are dispersed in Canada, they can moult during the summer and, if infected, transmit Lyme disease spirochetes to suitable vertebrate hosts. Additionally, *I. scapularis* immatures can act as a bridge vector to people and certain domestic animals. It is an inescapable fact that migratory songbirds are transporting ticks into Canada during northward spring migration, and some of these engorged ticks are infected with *B. burgdorferi* s.s., which is pathogenic to humans and certain domestic animals. Ultimately, it is firmly established that people do not have to frequent an endemic area to contract Lyme disease. Medical professionals, veterinarians, biomedical scientists, and public health officials should be vigilant that diverse variants of *B. burgdorferi* s.l. are introduced by songbird-

transported *Ixodes* ticks, and pose a country-wide public health risk.

Acknowledgments

We thank Simon Duval, Charlotte England, and Nigel Shaw for collecting ticks from migratory songbirds. We are indebted to Kenny Lou for technical assistance. We pay special tribute to the late Bronwyn Dalziel for her outstanding ornithology work. We are grateful to John Ward for computer graphics. Funding support was provided in part by Lyme Ontario.

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