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Research Paper

# Detection of Lyme Disease Bacterium, *Borrelia burgdorferi* sensu lato, in Blacklegged Ticks Collected in the Grand River Valley, Ontario, Canada

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## Abstract

We document the presence of blacklegged ticks, *Ixodes scapularis*, in the Grand River valley, Centre Wellington, Ontario. Overall, 15 (36%) of 42 *I. scapularis* adults collected from 41 mammalian hosts (dogs, cats, humans) were positive for the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.). Using real-time PCR testing and DNA sequencing of the flagellin (*fla*) gene, we determined that *Borrelia* amplicons extracted from *I. scapularis* adults belonged to *B. burgdorferi* sensu stricto (s.s.), which is pathogenic to humans and certain domestic animals. Based on the distribution of *I. scapularis* adults within the river basin, it appears likely that migratory birds provide an annual influx of *I. scapularis* immatures during northward spring migration. Health-care providers need to be aware that local residents can present with Lyme disease symptoms anytime during the year.

Key words: Blacklegged tick, *Ixodes scapularis*, Lyme disease, *Borrelia burgdorferi*, Infection prevalence, Grand River valley.

## Introduction

The blacklegged tick, *Ixodes scapularis* Say (northern populations previously considered as *I. dammini*) (Acari: Ixodidae), is a blood-sucking ectoparasite that is indigenous to North America east of the Rocky Mountains. This tick species has been collected from songbirds (Passeriformes) from as far north and as far west as the municipality of Slave Lake, Alberta, Canada [1, 2]. In nature, *I. scapularis* is a vector of a diverse array of protozoan, viral, and bacterial pathogens that can cause virulent diseases in animals, including humans [3].

The Lyme disease spirochete, *Borrelia burgdorferi* sensu lato (s.l.), is typically carried and transmitted by several hard-bodied ticks (Acari: Ixodidae) [4]. Globally, the *B. burgdorferi* s.l. complex consists of at least 23 genospecies and genomospecies. In North America, at least 9 *B. burgdorferi* s.l. genospecies are characterized, namely *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s.), *B. californiensis*, *B. carolinensis*, *B. garinii*, *B. kurtenbachii*, and *B. mayonii* [5-13]. Of these genospecies, *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* s.s., *B. garinii*, *B. kurtenbachii*, and *B. mayonii* are pathogenic to humans [14-17]. World-wide, Lyme disease has been diagnosed in over 80 countries [18].

In March 1995, an engorged *I. scapularis* female was collected from an untravelled canine living at Elora, Ontario; this represents the first record of this tick species in Centre Wellington (JDS). In the same locality, a *B. burgdorferi* s.l.-infected *I. scapularis* female was collected from a feral feline in 1999; this is the first record of a blacklegged tick positive for the Lyme

disease bacterium in this vicinity [19]. Human Lyme disease cases have been reported in Centre Wellington dating back to 1989. During a 16-year period (2001-2016), an accumulated total of 27 people have contracted Lyme disease in Centre Wellington.

Biogeographically, *B. burgdorferi*-positive *I. scapularis* larvae and nymphs have been collected from migratory songbirds during northward spring migration at Ruthven Park, which is situated on the Grand River south of Centre Wellington [20-22]. These larval and nymphal *I. scapularis* likely originate from the southern fringe of Canada or from the central and eastern United States where 27-47% of the *I. scapularis* adults are positive for *B. burgdorferi* s.l. [23, 24]. Of note, Scott et al. [1] reported an *Ixodes baergi* tick on a American Cliff Swallow, *Petrochelidon pyrrhonota*, collected at Elora, Ontario.

During northward spring migration, passerine migrants import *I. scapularis* larvae and nymphs into Canada from as far south as Florida and northeastern Mexico [25]. In addition, Neotropical and southern temperate passerine migrants can transport several species of *Ixodes* species (i.e., *I. affinis*, *I. brunneus*, *I. dentatus*, *I. minor*) and *Amblyomma* species (i.e., *A. americanum*, *A. dissimile*, *A. longirostre*, *A. maculatum*, *A. rotundatum*) from southern latitudes, and disperse them across Canada [1, 2, 18-22, 26]. All of these tick species can harbour tick-associated pathogens that cause diseases in humans and domestic animals [3, 27]. In particular, blacklegged ticks can harbour and transmit a wide array of tick-associated pathogens. These zoonotic pathogens include: *Babesia* spp. (e.g., *B. duncani*, *B. microti*), *Bartonella* spp. (e.g., *B. henselae*), *Ehrlichia* spp. (e.g., *E. ewingii*), *Mycoplasma* spp. (e.g., *M. fermentans*) [28], *Anaplasma* spp. (e.g., *A. phagocytophilum*), *Borrelia miyamotoi* (relapsing fever group spirochete), *Ehrlichia muris*-like agent, and Deer Tick Virus (Powassan virus group) [29]. Of epidemiological importance, *I. scapularis* can pass the human pathogen, *B. miyamotoi* via transovarial transmission to eggs and, subsequently, to larvae [30, 31].

When ground-foraging songbirds make landfall at an established population of *I. scapularis*, they can become parasitized by *I. scapularis* larvae and nymphs. Since there can be upwards of 1,000 *I. scapularis* larvae questing at an oviposition site, a ground-frequenting songbird can become heavily infested with larvae. Because *I. scapularis* immatures acquire blood meals at different rates, fully engorged ticks can drop off at various stopover locations along the migratory flight path. Stopovers are often located in forested areas, such as ecotones (woods' edge). These wooded corridors commonly follow rivers. Heavily infested songbirds can drop numerous *I. scapularis* larvae and nymphs in tick-conducive habitats, and start new Lyme disease foci [32, 33].

The purpose of this study was to determine the infection prevalence of *B. burgdorferi* s.l. in *I. scapularis* ticks collected from cats, *Felis catus*; dogs; *Canis lupus familiaris*; and humans, *Homo sapiens*, residing within the Grand River valley, which runs through Centre Wellington, and to ascertain if these ticks are a public health risk.

## Materials and Methods

A 3.5-year, tick-host study (2013-2016) was conducted in the Grand River valley between Inverhaugh, Ontario (43º 38′ 20″ N, 80º 26′ 21″ W) and Belwood, Ontario (43º 47′ 31″ N, 80º 19′ 22″ W). The river basin transects Centre Wellington, and encompasses urban municipalities and agricultural farmland (Figure 1). The predominant coniferous species are Eastern white cedar, *Thuja occidentalis*, and Norway spruce, *Picea abies*. The most prevalent deciduous tree species, which are native to the area, consist of: sugar maple, *Acer saccharum*; Manitoba

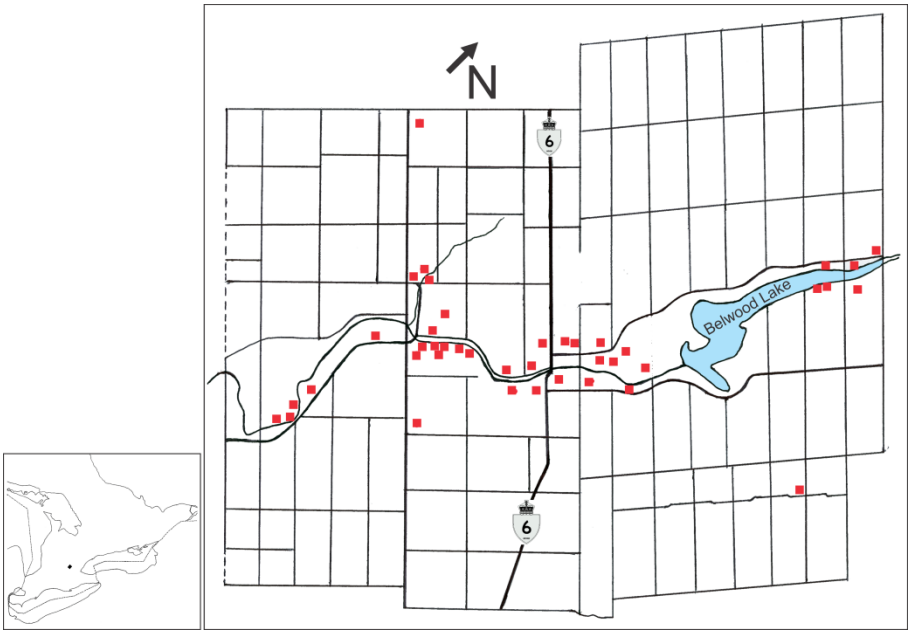


**Figure 1.** Map of Centre Wellington study area where *Ixodes scapularis* ticks were collected.

maple, *Acer negundo*; black walnut, *Juglans nigra*; silver maple, *Acer saccharinum*; and bur oak, *Quercus macrocarpa*.

The Grand River valley supports a wide range of wildlife mammals that are found commonly in deciduous forests in northeastern North America. The most prevalent large mammal is the white-tailed deer, *Odocoileus virginianus*. Although they are not competent reservoirs of *B. burgdorferi* s.l., they support *I. scapularis* reproduction [34]. Mid-sized mammals include eastern gray squirrel, *Sciurus carolinesis*; red squirrel, *Tamiasciurus hudsonicus*; eastern cottontail rabbit, *Sylvilagus floridanus*; raccoon, *Procyon lotor*; and striped skunk, *Mephitis mephitis*. Small mammals include deer mice, *Peromyscus maniculatus*; meadow vole, *Microtus pennsylvanicus*; northern short-tailed shrew, *Blarina brevicauda*; eastern chipmunk, *Tamias striatus*; and house mouse, *Mus musculus*.

### Tick collection

Veterinarians and local residents provided ticks that were obtained from mammalian hosts that included cats, dogs, and people. As a tick guide, we prepared and distributed a colored chart showing four species of ticks, which have been identified in the area. These tick species include the blacklegged tick, groundhog tick (*Ixodes cookei*), American dog tick (*Dermacentor variabilis*), and lone star tick (*Amblyomma americanum*). Each veterinarian was given a pad of 'Tick-Host Information' sheets, and asked to complete and submit one with each tick specimen. The background information included: host, location/residency of companion animal, travel history, date collected, and collector. Animals with a history of travel were excluded from the study. Veterinarians removed ticks with fine-point, stainless steel tweezers, and placed them in round-bottom, 8.5 mL polypropylene tubes (15.7 X 75 mm) labelled with background information. A 7-mm hole was drilled in the polyethylene push caps (15.7 mm diameter) for ventilation. To prevent ticks from escaping, tulle netting was placed over the mouth of the vial before the push cap was inserted. The capped vials were then placed in self-sealing, double-zipper, plastic bags with a section of slightly moistened paper towel. Ticks were directly delivered to the tick identification laboratory (JDS) for examination and recording. Ticks were identified morphologically using taxonomic keys [35-37].

Flagging was done at one location where individual *I. scapularis* females were collected from the same dog for 2 consecutive years.

In order to check the winter hardiness of *I. scapularis*, we placed 10-20 adults in a deciduous woodlot each fall (2013, 2014, 2015), and collected them the following spring. They were placed in vented polyethylene vials, which were inserted into a vented, plastic canister (63 mm × 135 mm). This container was covered with aluminum hardware screen for mouse exclusion. The screened canister was then put in an open-ended wooden crate (80 mm × 125 mm × 150 mm) for cervid hoof protection. A layer of deciduous leaves was placed over the overwinter box to reflect the surrounding leaf layer.

We examined bird-tick data obtained during a 6-year period (2011-2016) in southwestern Ontario; ticks were collected from songbirds in the lower Grand River valley, which is directly south of our study area.

### Spirochete detection

In order to undertake *B. burgdorferi* s.l. testing, we had 3 separate delivery phases: phase 1 (3 May 2013 to 31 May 2014), phase 2 (26 October 2014 to 7 May 2015), and phase 3 (7 June 2015 to 7 June 2016). In phase 1, ticks were sent by courier to a laboratory (JFA) for culturing and PCR amplification. Live ticks were cultured, and these ticks and dead ticks were later tested for *B. burgdorferi* s.l. using DNA extraction and PCR amplification of the highly-conserved, outer surface protein A (*OspA*) gene. The DNA detection methods have been previously described [38-40]. During phase 2, ticks were put in 2 mL micro tubes containing 94% ethyl alcohol, and sent by courier to a separate laboratory (KLC) for *B. burgdorferi* s.l. testing and molecular analysis. DNA extraction and PCR testing using the flagellin B (*flaB*) gene were performed as previously described [41]. For phase 3, each tick was put in a 2 mL micro tube containing 94% ethyl alcohol, and sent by courier to the PCR amplification laboratory (JEF). The laboratory procedures, including DNA extraction, PCR testing, DNA sequencing, and amplicon evaluation were conducted as previously described [42]. The flagellin (*fla*) gene was amplified.

### Nucleotide sequences

DNA sequences of the *fla* gene of *B. burgdorferi* s.s. amplicons were deposited in the GenBank database with accession numbers: KX085197 for tick 15-5A84A, KX085198 for tick 15-5A84B, KX085199 for 15-5A100, and KX085200 for tick 15-5A106.

## Results

### Tick collection

During the 3.5-year study (May 2013 to August 2016), 42 *I. scapularis* ticks were collected from 41 mammalian hosts (4 cats, 29 dogs, 8 humans) in the Grand River valley (Table 1) (Figure 1, 2). We collected four other species of ticks in the Grand River

corridor, namely *A. americanum*, *A. maculatum* (Gulf Coast tick), *D. variabilis*, and *I. cookei*. Of special note, a fully engorged *A. americanum* female was collected on 19 August 2013 from a dog with no history of travel. Likewise, a slightly engorged *A. maculatum* female was collected from a dog with no history of travel on 27 August 2016. These *Amblyomma* ticks were likely imported by migratory songbirds as nymphs during northward spring migration and, subsequently, molted to females during the summer.

When we conducted flagging where individual *I. scapularis* females had been collected from the same dog for 2 consecutive years, we were unable to find any more *I. scapularis* adults.

When the *I. scapularis* adults, which were placed in local wooded area to overwinter, where checked in the spring, the overwinter survival rate was 95% (range, 90-100%).

**Table 1**. Presence of *Borrelia burgdorferi* s.l. in *Ixodes scapularis* adults collected from mammalian hosts residing in the Grand River valley, 2013-2016

| Host | No. of hosts | No. of ticks collected | No. of ticks PCR pos. (%) |
|---|---|---|---|
| Cat, *Felis catus* | 4 | 4 | 1 (25) |
| Dog, *Canis lupus familiaris* | 29 | 30 | 10 (33) |
| Human, *Homo sapiens* | 8 | 8 | 4 (50) |
| Total | 41 | 42 | 15 (36) |

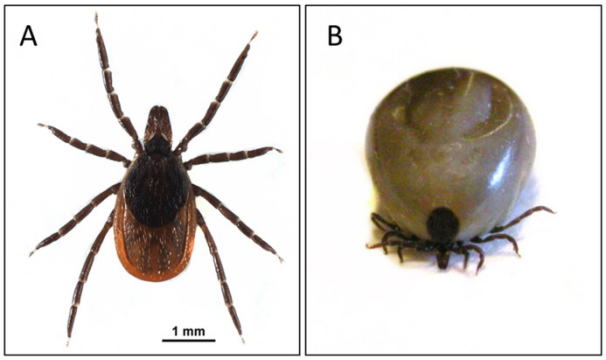


**Figure 2.** *Ixodes scapularis*: A. unfed female; and B. fully engorged female, body length: 9.3 mm. Photo credits: Elizabeth J. Sears.

### Spirochete detection

In total, 15 (36%) of 42 *I. scapularis* adults, were infected with *B. burgdorferi* s.l. (Table 1). Based on DNA sequencing of the flagellin (*fla*) gene of *Borrelia* amplicons from four ticks (15-5A84A, 15-5A84B, 15-5A100, 15-5A106), we determined that they belong to *B. burgdorferi* s.s., which is pathogenic to people and certain domestic animals. Interestingly, one dog was co-infested with two *I. scapularis* females, and the DNA sequences of the *B. burgdorferi* s.l. amplicons were slightly different. This molecular phenomenon suggests that these two co-feeding ticks had fed on two different vertebrate sources, as larvae or nymphs, before the resulting *I. scapularis* females parasitized the host dog. Amplicons of *B. burgdorferi* s.l. from four *I. scapularis* adults were DNA sequenced, and characterized as *B. burgdorferi* s.s. Of 5 *I. cookei* (2 females, 3 nymphs) tested, one nymph was positive for *B. burgdorferi* s.l.; it was collected from an adult human female. Even though many of the ixodid ticks were PCR-negative for Lyme disease spirochetes, they could have been infected with a wide diversity of other tick-transmitted pathogens. Certain tick species (i.e., *A. americanum*, *A. maculatum*, *D. variabilis*) were not tested for *B. burgdorferi* s.l.

In 1993, fifteen small mammals (8 deer mice, 5 northern short-tailed shrews, 1 eastern chipmunk, 1 red squirrel) were collected in the area encompassing Fergus, Ontario; 2 northern short-tailed shrews were PCR-positive for *B. burgdorferi* s.l. As well, spirochetes were observed in organs (ear, heart, kidney, spleen, liver, bladder) of these shrews using anti-*B. burgdorferi* s.l. antibody fluorescent stain and dark-field microscopy (S. N. Banerjee, unpublished data).

During a 6-year period (2011-2016), we collected 34 *I. scapularis* immatures (32 nymphs, 2 larvae) from songbirds in the lower Grand River riverine corridor. Of these ticks, 11 (34%) of 32 *I. scapularis* nymphs were infected with *B. burgdorferi* s.l.

## Discussion

We document a 36% infection prevalence of *B. burgdorferi* s.l. in *I. scapularis* adults collected from dogs, cats, and people in the upper Grand River valley. Additionally, we provide circumstantial evidence that migratory songbirds introduce *I. scapularis* nymphs during spring migration, and drop them randomly along the Grand River riverine corridor. Most importantly, these *B. burgdorferi* s.l.-infected *I. scapularis* pose a public health risk to people and domestic animals.

### Epidemiology of Lyme disease in Grand River valley

Lyme disease has been present in the Grand River valley for decades. The first reported case of Lyme disease in Centre Wellington was in 1989; an infected human adult male with no history of travel was recognized. In 1993, a small mammal study was conducted in the Fergus area, and northern short-tailed shrews were PCR-positive for *B. burgdorferi* s.l. In addition, Lyme disease spirochetes

were observed in tissues of these shrews using dark-field microscopy and anti-*B. burgdorferi* s.l. antibody fluorescent stain. During a 10-yr, tick-host study [19], an engorged *I. scapularis* female was collected from an untraveled dog at Elora, Ontario on 10 March 1995; this tick constitutes the first *I. scapularis* tick reported in Centre Wellington. A fully engorged *I. scapularis* female was collected from an untravelled cat, which was living near Alma on 18 December 1999. Not only was the attached *I. scapularis* tick positive for *B. burgdorferi* s.l., the host cat was serologically positive and symptomatic for Lyme disease. Of epidemiological significance, 4 (50%) of 8 *I. scapularis* adults on humans were infected with *B. burgdorferi* s.l. These tick-host-pathogen findings clearly show that *I. scapularis* ticks have been present in the Grand River corridor for decades, and have caused Lyme disease in local residents.

### Prevalence of *B. burgdorferi* s.l. in *I. scapularis*

Our findings reveal a 36% infection prevalence for *B. burgdorferi* s.l. in *I. scapularis* adults collected in the Grand River valley. This infection prevalence is within the range reported in other studies in the upper Midwest and the northeastern United States, which range from 27-47% [20, 21]. Similarly, Scott et al. [43] reported a *B. burgdorferi* s.l. infection prevalence of 41% in *I. scapularis* adults collected recently in the Hamilton-Wentworth, Ontario region. Unlike *B. miyamotoi*, transovarial transmission of *B. burgdorferi* s.l. from *I. scapularis* females to larvae does not occur [31]. Therefore, any *I. scapularis* larvae, which are infected with *B. burgdorferi* s.l., must have acquired infection from spirochetemic mammals in Centre Wellington or from reservoir-competent, migratory songbirds.

### Absence of *I. scapularis* nymphs

During the study period, *I. scapularis* nymphs were not submitted or collected. These findings are consistent with a 10-yr, tick-host study (1993-2002) conducted with veterinary clinics across Ontario [19]. On the other hand, *I. cookei* nymphs were collected from domestic animals and people. This phenomenon suggests that *I. cookei* is established locally, whereas *I. scapularis* is not. The absence of *I. scapularis* nymphs on domestic animals and people in this locality suggests that this tick is introduced annually by passerine migrants. Bird parasitism by *I. scapularis* immatures is present in the Grand River riverine corridor [22-24]. A bird bander, who is located at a more southerly location along the Grand River, has collected *I. scapularis* immatures during northward spring migration. When these I. *scapularis* nymphs are released at more northerly locations in Canada, the *B. burgdorferi* s.l. infection prevalence stays the same in adults. Notably, this infection prevalence (34%) has been reflected in Centre Wellington in *I. scapularis* adults.

### Tick dispersal by birds

Songbirds play a significant role in the wide dispersal of bird-feeding ticks during northward spring migration. As passerine migrants fly north to seasonal breeding grounds, they are parasitized by Lyme disease vector ticks when they make landfall and, a few days later, these replete ticks drop to the ground at more northerly stopover sites. Notably, bird-fed *I. scapularis* nymphs molt in 5-9 wk, and typically develop to adults from late June to early August. When recently moulted *I. scapularis* adults become sclerotized and their reproductive organs are fully developed, they typically start questing in Ontario for hosts in October. If they fail to parasitize a host, they overwinter during frigid temperatures under an insulating blanket of snow [44], and start host-seeking in the spring once the snow has disappeared. Since *I. scapularis* ticks have antifreeze-like compounds (glycoproteins) in their bodies [45], they are able to withstand subzero temperatures. In the present study, the overwinter survival of *I. scapularis* adults was 95%. Therefore, people must be vigilant that *I. scapularis* ticks are actively engaged in host-seeking activities in Ontario when the snow disappears in the spring until sustained snowfall in the late fall.

The prevalence of *B. burgdorferi* s.l. (36%) in *I. scapularis* adults from mammalian hosts in our study is consistent with the prevalence of Lyme disease spirochetes in *I. scapularis* nymphs (35%) collected from songbirds in central and eastern Canada [22]. This phenomenon provides circumstantial evidence that migratory songbirds are the source of *I. scapularis* ticks in the Grand River riverine corridor. Furthermore, Morshed et al. [19] found that all of the *I. scapularis* ticks collected from animals during a 10-year, tick-host study conducted across Ontario were adults, which implies wide dispersal of ticks by songbirds.

In the present study, an *A. americanum* female was collected from an untravelled dog in mid-summer. Since *A. americanum* ticks are not indigenous to Canada, it is most likely that a southern temperate or Neotropical songbird transported this *A. americanum* specimen, as a nymph, to the Grand River area during northward spring migration [1]. Subsequently, after the nymph dropped into the cool, moist leaf litter, and molted to a female in 6-10 wk, it parasitized the dog in August. In addition, the collection of a Gulf Coast tick on an untravelled dog in

late August reveals that passerine migrants are also implicated in the importation of this tick into Canada. Since *A. maculatum* is not indigenous to Canada, this tick, in all likelihood, was transported to the area by a southern temperate or Neotropical songbird during spring migration and, during the summer, molted to a female. Biogeographically, *A. maculatum* immatures have been collected in Canada from migratory songbirds during northward spring migration [1, 18, 26, 35]. The collection of an *A. maculatum* female in the Grand River area provides further evidence that migratory songbirds are transporting bird-feeding ticks into southern Canada. Notably, *Amblyomma* species can harbour a wide array of tick-borne pathogens that can be transmitted to domestic animals and people [3].

Migratory songbirds can transport bird-derived ticks hundreds of kilometres into Canada during annual spring migration from as far south as the southern United States, the Caribbean, and Central and South America [1, 2, 18-22, 26, 46-48]. Not only do long-distance migrants provide an influx of *I. scapularis* immatures, they contribute to the establishment of Lyme disease foci [33, 49]. Since passerine migrants widely disperse Lyme disease vector ticks, people do not have to visit an endemic area to contract Lyme disease.

## Diversity of *B. burgdorferi* s.l.

In the present study, two *I. scapularis* females were collected from a single, untraveled dog on the same day. Surprisingly, these females harbored slightly different strains of *B. burgdorferi* s.l. The genetic difference of *B. burgdorferi* s.l. in these two ticks, was a single nucleotide substitution. Even though these two ticks were co-feeding on the same dog, the genetic variation suggests that these ticks originated from different geographic sources. It is noteworthy that one *I. scapularis* immature could have parasitized a songbird at one stopover, whereas another passerine migrant was parasitized at another stopover. Alternatively, a songbird could have become co-infected with two different strains of *B. burgdorferi* s.l., and transmitted them to co-feeding nymphal *I. scapularis* [50]. Since passerine migrants make landfall at different Lyme disease foci along the migratory flight path, they can introduce a wide diversity of ticks and Lyme disease spirochetes into the Grand River corridor. Richter et al. [51] reveal that the American Robin, *Turdus migratorius*, is a reservoir-competent host of *B. burgdorferi* s.l. Furthermore, *B. burgdorferi* s.l. has been isolated from the blood and skin of passerines [52-55]. Moreover, *B. burgdorferi* s.l. has been detected in songbird-derived *I. scapularis* larvae, which indicates that many passerines are reservoirs of Lyme disease spirochetes [21, 22, 53, 56]. Migratory songbirds are known to transport *I. scapularis* immatures with more than one tick-borne pathogen. For example, a nymphal *I. scapularis* was collected from a Veery, *Catharus fuscescens*, co-infected with *B. burgdorferi* s.l., *Babesia microti* (human babesiosis), and *Anaplasma phagocytophilum* (human anaplasmosis) [57]. In Michigan, Hamer et al. [58] reported *Borrelia andersonii* and, likewise, *B. miyamotoi* in *I. dentatus*, and *I. scapularis* collected from passerine birds. Additionally, *B. andersonii* has been detected in patients in the southeastern United States [15]. Not only do songbirds act as reservoirs of *B. burgdorferi* s.l., they are disseminators of borreliae and other tick-borne pathogens.

## Clinical and Zoonotic Implications

Our study reveals that *I. scapularis* ticks collected in the Grand River valley harbour *B. burgdorferi* s.s., which is pathogenic to humans. This microorganism causes Lyme disease, a multisystem spirochetosis. As this zoonotic disease progresses, patients commonly experience fatigue, joint pain, neurologic symptoms, and cognitive dysfunction. When a *B. burgdorferi* s.l.-infected *I. scapularis* tick takes a blood meal, it can transmit spirochetes and, if co-infected, it can transmit one or more of at least 8 tick-transmitted, pathogenic microorganisms. During *I. scapularis* attachment, *B. burgdorferi* s.l. can be transmitted in less than 24 hours, especially if the tick salivary glands are infected [59]. During the initial phase of cutaneous infection following a tick bite, patients may have an erythema migrans rash that may be bull's-eye shaped or atypical; however, less than 42% of patients have these rashes [60, 61]. Therefore, when someone is bitten by an *I. scapularis* tick, that individual should seek medical attention and prophylactic antimicrobial treatment.

In humans, *B. burgdorferi* s.l. has diverse forms (i.e., spirochetes, blebs, granules, round bodies, atypical forms). Spirochetes shape-shift into round bodies and hide in gelatinous shields called biofilms allowing this highly adaptable microbe to become a stealth pathogen. In addition, spirochetes will attach to, invade, and kill human B and T lymphocytes [62]. As spirochetes spread in the body, a multitude of clinical manifestations can unfold, including fatigue, headaches, low-grade fever, stiff neck/back, disturbed sleep, memory loss and cognitive dysfunction. Migratory joint aches and nerve inflammation with numbness and tingling are typical symptoms. This illness can affect several body systems: cardiac, endocrine, gastrointestinal, musculoskeletal, neurological, ontological, and

ophthalmological [63]. Neurological deficits are common in both children and adults. As spirochetes attack nerves and ligamentous tissue, they produce neurotoxins that cause an inflammatory response in the surrounding tissue [64, 65]. As the spirochetemia progresses in the central nervous system, demyelination may occur with cytokine dysregulation and profound fatigue [65, 66].

Left untreated or inadequately treated, *B. burgdorferi* s.l. will hide in deep tissues, such as bone [67], brain [68-70], eye [71], muscle [72], collagenous tissues (ligaments, tendons) [73, 74], glial and neuronal cells [75, 76], and fibroblast/scar tissue [77]. Lack of response to treatment can be due to persistent infection caused by spirochete sequestration in tissues or biofilm formation. In some cases, this persistent infection is fatal [68, 78]. Since *B. burgdorferi* can be persistent, Lyme disease spirochetes have been detected in and cultured from tissues and body fluids after conventional, short-term antibiotic treatment in animals and humans [79-84]. Since spirochetes lodge in human testicles, semen, and vaginal secretions, *B. burgdorferi* s.l. may be sexually transmitted [85, 86]. Lyme disease can destroy people's lives, and *B. burgdorferi* s.l. infection should be considered among the differential diagnoses for patients who have signs and symptoms suggestive of tick-borne illness.

In conclusion, our findings underpin the fact that multiple species of ticks are present in the Grand River valley, and 36% of the *I. scapularis* adults are infected with *B. burgdorferi* s.l. The close similarity of the *B. burgdorferi* s.l. infection prevalence of *I. scapularis* adults on mammalians hosts, including people, and the infection prevalence of *I. scapularis* nymphs in eastern and central Canada indicates that migratory songbirds are introducing *B. burgdorferi* s.l.-infected ticks into Centre Wellington annually. Health-care providers need to be aware that *I. scapularis* ticks infected with pathogenic *B. burgdorferi* s.s. are present in the Grand River valley, and present a public health risk.

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## Competing Interests

The authors confirm that they have no conflicts of interest that could bias any aspect of the present paper. They have no financial interests in, relationships with, or have received no funding from any clinical laboratories or test kit companies or reagent manufactures mentioned in the paper.

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