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Authors

Scott, John D
Foley, Janet E
Clark, Kerry L
et al.

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Research Paper

Established Population of Blacklegged Ticks with High Infection Prevalence for the Lyme Disease Bacterium, *Borrelia burgdorferi* Sensu Lato, on Corkscrew Island, Kenora District, Ontario

John D. Scott[1]✉, Janet E. Foley[2], Kerry L. Clark[3], John F. Anderson[4], Lance A. Durden[5], Jodi M. Manord[3], Morgan L. Smith[3]

1. Lyme Ontario, Research Division, 365 St. David St. South, Fergus, Ontario, Canada N1M 2L7;
2. Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616, United States of America;
3. Epidemiology & Environmental Health, Department of Public Health, University of North Florida, 1 UNF Drive, Jacksonville, Florida 32224, United States of America;
4. Department of Entomology and Center for Vector Ecology and Zoonotic Diseases. The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504-1106, United States of America;
5. Department of Biology, Georgia Southern University, 4324 Old Register Road, Statesboro, Georgia 30458, United States of America.

✉ Corresponding author: 365 St. David Street South, Ontario, Canada N1M 2L7. Telephone: 519-843-3646; Fax: 519-843-6550; e-mail: jkscott@bserv.com

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Abstract

We document an established population of blacklegged ticks, *Ixodes scapularis*, on Corkscrew Island, Kenora District, Ontario, Canada. Primers of the outer surface protein A (*OspA*) gene, the flagellin (*fla*) gene, and the flagellin B (*flaB*) gene were used in the PCR assays to detect *Borrelia burgdorferi* sensu lato (s.l.), the Lyme disease bacterium. In all, 60 (73%) of 82 adult *I. scapularis*, were infected with *B. burgdorferi* s.l. As well, 6 (43%) of 14 unfed *I. scapularis* nymphs were positive for *B. burgdorferi* s.l. An *I. scapularis* larva was also collected from a deer mouse, and several unfed larvae were gathered by flagging leaf litter. Based on DNA sequencing of randomly selected *Borrelia* amplicons from six nymphal and adult *I. scapularis* ticks, primers for the flagellin (*fla*) and flagellin B (*flaB*) genes reveal the presence of *B. burgdorferi* sensu stricto (s.s.), a genospecies pathogenic to humans and certain domestic animals. We collected all 3 host-feeding life stages of *I. scapularis* in a single year, and report the northernmost established population of *I. scapularis* in Ontario. Corkscrew Island is hyperendemic for Lyme disease and has the highest prevalence of *B. burgdorferi* s.l. for any established population in Canada. Because of this very high infection prevalence, this population of *I. scapularis* has likely been established for decades. Of epidemiological significance, cottage owners, island visitors, outdoors enthusiasts, and medical professionals must be vigilant that *B. burgdorferi* s.l.-infected *I. scapularis* on Corkscrew Island pose a serious public health risk.

Key words: blacklegged tick, *Ixodes scapularis*, Lyme disease, *Borrelia burgdorferi*, infection prevalence, Kenora District, Ontario

Introduction

The blacklegged tick, *Ixodes scapularis* (northern populations previously treated as *I. dammini*) (Acari: Ixodidae), is the principal North American vector of the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.) east of the Rocky Mountains [1]. In northern latitudes, *I. scapularis* typically has a 2-yr life cycle that consists of egg, larva, nymph, and adult (male, female), and has a diapause in the winter months throughout northwestern Ontario. Worldwide, the *B. burgdorferi* s.l. complex comprises of at least 23 genospecies or genomospecies. In North America, at least 10 *B. burgdorferi* s.l. genospecies/genomospecies are present, namely *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s.), *B.*

californiensis, *B. carolinensis*, *B. garinii*, *Borrelia* genomospecies 2, *B. kurtenbachii*, and *B. mayonii* [2-10]. Of these genospecies, *B. americana*, *B. andersonii*, *B. bissettii*, *B. burgdorferi s.s.*, *B. garinii*, *B. kurtenbachii*, and *B. mayonii* are known to be pathogenic to humans and certain domestic animals [9, 11-14].

Blacklegged ticks feed on more than 125 North American vertebrates (avian, mammalian, reptilian) [15]. This ixodid tick has been collected from at least 81 bird species in the United States and Canada and, in particular, songbirds (Passeriformes) play a key role in the wide dispersal of *I. scapularis* larvae and nymphs. Biogeographically, larval and nymphal *I. scapularis* have been reported during spring migration on Neotropical songbirds as far north and as far west as Slave Lake, Alberta [16, 17]. As well, *I. scapularis* immatures have been recorded on passerine migrants in Saskatchewan, Manitoba, northern Ontario, southern Ontario, Quebec, New Brunswick, Nova Scotia, and Prince Edward Island [16-21]. Pertinent to the present study, passerine migrants provide an influx of bird-feeding ticks annually to the Kenora District.

Historically, Banerjee et al. [22] isolated *B. burgdorferi* s.l. from an *I. scapularis* female collected from a resident dog of Kenora, Ontario with no history of travel. Subsequently, Canadian tick researchers reported *B. burgdorferi* s.l.-positive *I. scapularis* on people and domestic hosts residing between Kenora and Clearwater Bay, and further north in the Kenora District [23]. In the upper Midwest, Turtinen et al. [24] reported an infection prevalence of 35.7% for *B. burgdorferi* s.l. in *I. scapularis* adults collected in Wisconsin.

The aim of this study was to determine if there is an established population of *I. scapularis* on Corkscrew Island and to determine the prevalence of *B. burgdorferi* s.l. in these ticks.

Materials and Methods

Study area. Corkscrew Island, Ontario (49º 40′ 36″ N, 94º 40′ 58″ W) is located in the northern part of Lake of the Woods between Clearwater Bay and Kenora, Ontario (Figure 1). This 1064.7 ha, zigzag-shaped island is situated along the southern fringe of the Canadian Shield, which consists of Precambian igneous rock, and lies within the southernmost belt of the boreal forest. Geographically, this insular tract of land is 1.5 km from the mainland (on the east side). A grassy meadow extends over part of the core area, while a deciduous-coniferous forest covers much of the perimeter of the island. The predominant tree species include trembling aspen, *Populus tremoides*; bur oak, *Quercus macrocarpa*; red ash, *Fraxinus pennsylvanica*; black ash, *Fraxinus nigra*; white spruce, *Picea glauca*; black spruce, *Picea mariana*; and eastern white pine, *Pinus strobus*. Smaller arboreal shrubs include: American hazelnut, *Corylus americana*; Saskatoon berry, *Amelanchier alnifolia*; bittersweet, *Celastrus scandens*; and smooth rose, *Rosa blanda*. Poison ivy, *Rhus radicans*, is prevalent, and various grass species abound, especially in the central area of the island.

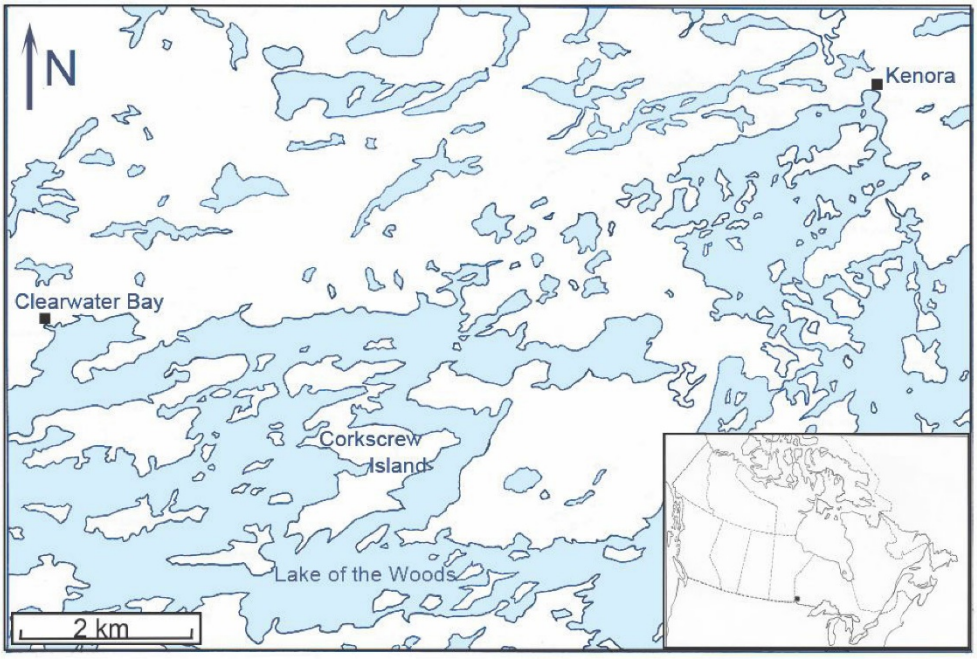

Figure 1. Map of the northern part of Lake of the Woods showing the geographic location of Corkscrew Island, Kenora District, Ontario.

Large animals consist of white-tailed deer, *Odocoileus virginianus*; American black bear, *Ursus americanus*; and gray wolf, *Canis lupus*. Medium-sized animals include Canadian beaver, *Castor canadensis*; red fox, *Vulpes vulpes*; raccoon, *Procyon lotor*; and snowshoe hare, *Lepus americanus*. Small mammals comprise: deer mouse, *Peromyscus maniculatus*; meadow vole, *Microtus pennsylvanicus*; southern red-backed vole, *Myodes gapperi*; northern short-tailed shrew, *Blarina brevicauda*; eastern chipmunk, *Tamias striatus*; least chipmunk, *Tamias minimus*; and American red squirrel, *Tamiasciurus hudsonicus*.

Gallinaceous birds include Ruffed Grouse, *Bonasa umbellus* and Spruce Grouse, *Falcipennis canadensis*, whereas some of the prominent ground-foraging passerines include Song Sparrow, *Melospiza melodia*; Pine Grosbeak, *Pinicola enucleator*; Eastern Phoebe, *Sayornis phoebe*; and Blue Jay, *Cyanocitta cristata*.

Tick collection. Blacklegged tick adults were collected by flagging low-level vegetation during the spring and fall bimodal questing periods (spring 2014 to spring 2016) (Figure 2A, B). Nymphs were collected from the leaf litter by flagging around bur oaks during late May and early June. The habitats for flagging included open field (grass meadow), ecotone (woods edge), and open canopy (sparse trees). The flag cloth was made from a piece of sweatshirt fleece measuring 70 cm by 80 cm. Ticks were removed from the flag with fine-pointed tweezers, and put in 8.5 mL polypropylene vials (15.7 mm × 74 mm) with a label listing background information (i.e., geographical location, date collected). A 7-mm hole in the polyethylene push-cap (15.7 mm diameter) provided ventilation for the ticks. After the ticks were inserted, a piece of tulle netting was placed over the mouth of the vial before inserting the push-cap preventing ticks from escaping. The vial was placed in a self-sealing, double-zippered plastic bag with a slightly moistened section of paper towel, and sent in a bubble-pack envelope to the laboratory (JDS). A taxonomic key and re-description information were employed for morphological identification [15, 25].

We flagged leaf litter within a radius of 3 m from the trunks of mature bur oaks in both open canopy and ecotone areas for nymphs during the nymphal questing period (28 May 2016 – 19 June 2016) (Figure 2C).

In order to check winter hardiness, we set out live *I. scapularis* adults in a wooded area in October (2015) and collected them in April (2016). They were placed in vented polyethylene vials that were inserted in a vented, plastic canister (63 mm × 135 mm). This container was covered with aluminum screen for mouse exclusion. The screened canister was then put in an open-ended wooden crate (80 mm × 125 mm × 150 mm) for hoof protection. A layer of leaves was placed over the overwinter box to reflect the surrounding leaf layer.

Spirochete detection. During Phase 1, we sent live ticks to the vector ecology and zoonotic diseases laboratory (JFA) for culturing. Live ticks were cultured in Barbour-Stoenner-Kelly (BSK) medium, and dead ticks were directly tested using DNA extraction and PCR testing. The DNA detection protocols have been described previously [26-28]. Although Persing et al. [26] used both the flagellin gene (*fla*) and the major outer surface protein A (*OspA*) gene, which is on the 49-kbp linear plasmid, we only employed the *OspA* gene in Phase 1 study. Appropriate negative and positive controls were used.

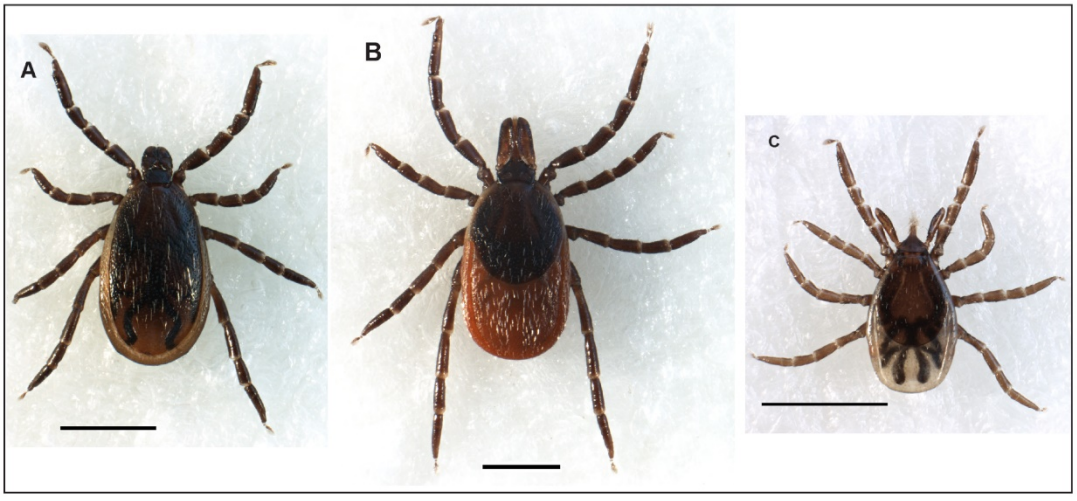

Figure 2. Blacklegged ticks, A) male, B) unfed female, and C) unfed nymph. Bar, 1 mm. Photo credit: Kellyn Hough

For Phase 2, ticks were put in 94% ethyl alcohol and forwarded to the environmental epidemiology research laboratory (KLC). These ticks were PCR tested using primers of the flagellin B (*flaB*) gene and the 16S-23S r RNA intergenic spacer gene. For ticks collected in the latter part of Phase 2, we only used the *flaB* gene. The methodology is described in Scott et al. [29]. In Phase 2, the negative control consisted of nuclease-free TE buffer. In order to prevent DNA contamination, a positive control sample was not used. Amplicons of the 194-bp (base position 313 to 506) and the 206-bp (base position 532 to 737) of the *B. burgdorferi* s.l. *flaB* gene were obtained from four *I. scapularis* adults (14-5A192A-1, 14-5A197, 14-5A201A, 15-5A79A) using PCR1 and PCR2 primer sets, respectively.

For Phase 3, ticks were sent by courier to the biomolecular laboratory (JEF). These ticks were PCR tested using primers of the flagellin (*fla*) gene to detect *B. burgdorferi* s.l., and the procedures are described elsewhere [30, 31].

The infection prevalence of *B. burgdorferi* s.l. in *I. scapularis* adults was calculated by dividing the total number of *B. burgdorferi* s.l.-infected ticks by the total number of *I. scapularis* males and females tested. Likewise, the same calculations apply to nymphs.

Nucleotide sequences. In phase 2, DNA sequences of the *flaB* gene of *B. burgdorferi* s.l. amplicons were deposited in the GenBank database with accession numbers: KT807493, KT827334 for tick 14-5A192-1; KT807495, KT827328 for tick 14-5A197; KT807496, KT827329 for tick 14-5A201A; and KX011448 for tick 15-5A79A. In phase 3, nucleotide sequences for the *fla* gene were obtained from an unfed nymph (16-5A36A) and an unfed female (16-5A10F4), and the GenBank accession numbers are KX459422 and KX459423, respectively.

Results

Tick collection. All host-feeding life stages (larvae, nymphs, adults) of *I. scapularis* were collected from Corkscrew Island. In total, 130 *I. scapularis* adults were gathered by flagging low-level vegetation during a 3-yr period (Figure 2A, B). In addition, we gleaned 15 unfed, questing *I. scapularis* nymphs from the forest floor by flagging leaf litter contiguous to bur oaks during the late spring (28 May to 19 June 2016) (Figure 2C). An *I. scapularis* larva was collected from a juvenile deer mouse, which captured in a domestic mouse trap on 3 September, 2016; several unfed, questing larvae were also obtained by flagging leaf litter around bur oaks in September and early October 2016.

In addition, an *I. scapularis* female was removed from an adult human female in mid-October 2013 and an *I. scapularis* male was detached from an adult human male in May 2016; these adult ticks were both attached to seasonal cottagers on Corkscrew Island.

We found that the ecotone and open canopy had the most *I. scapularis* ticks. During flagging, we found a close correlation between bur oak and questing *I. scapularis*. We estimate that 90% of the *I. scapularis* nymphs and adults were collected within 3 m of the trunks of bur oaks.

For the overwinter survival study (2015-2016), 13 (93%) of 14 *I. scapularis* males and females overwintered successfully in an outdoor wooded area (a single female died). Because we have collected *I. scapularis* adults, each spring, for 3 years, we have documented the overwintering of *I. scapularis* adults at this site for 3 consecutive winters.

A sample of 20 adult American dog ticks, *Dermacentor variabilis*, was collected but not tested for *B. burgdorferi* s.l. because this tick species is not a competent vector of Lyme disease spirochetes. Ecologically, we found that American dog ticks are sympatric with blacklegged ticks on Corkscrew Island. Two unfed nymphs of the rabbit tick, *Haemaphysalis leporispalustris*, were collected from the leaf litter by flagging in late spring. As well, several *H. leporispalustris* larvae were collected by flagging leaf litter in late summer.

Spirochete detection. Of 130 *I. scapularis* adults collected, 60 (73%) of 82 were positive for *B. burgdorferi* s.l. (Table 1). Overall, flagging was conducted for 18.0 hours, which averaged 7.2 *I. scapularis* males and females per hour (range, 3 to 28 adults/h). Using DNA sequencing, *B. burgdorferi* s.s. was characterized. A live culture of *B. burgdorferi* s.l. was obtained from one of the *I. scapularis* females (14-5A134B) during Phase 1 (JFA); however, it was not sent for DNA sequencing.

Table 1. Detection of *B. burgdorferi* s.l. in *I. scapularis* adults collected by flagging on Corkscrew Island, Ontario, 2014-2016

Collection period	No. of ticks tested	Ticks testing PCR-pos. (%)
Spring 2014	4	3 (75)
Fall 2014	15	12 (80)
Spring 2015	35	23 (66)
Fall 2015	10	7 (70)
Spring 2016	18	15 (83)
Total	82	60 (73)

PCR-pos., *Borrelia burgdorferi* s.l.-positive

Of the *I. scapularis* nymphs tested, 6 (43%) of 14 were positive for *B. burgdorferi* s.l. This infection prevalence is the highest ever reported for *I. scapularis* nymphs in Canada. Since transovarial transmission of *B. burgdorferi* s.l. in *I. scapularis* is not present, larvae

were not tested for *B. burgdorferi* s.l. The collection of all host-feeding stages (larva, nymph, adult) of *I. scapularis* underpins the presence of an established population of *I. scapularis* on Corkscrew Island. In addition, the two *H. leporispalustris* nymphs were tested for *B. burgdorferi* s.l., but were negative.

Discussion

Significant epidemiological findings. We document a hyperendemic area for Lyme disease on Corkscrew Island, and validate that *I. scapularis* ticks overwinter successfully on this island. At the same time, we report the most northern Lyme disease endemic area in Ontario. All three host-feeding life stages were collected in a single year, and these collections confirm an established population of blacklegged ticks. The infection prevalence for adult *B. burgdorferi* s.l. was 73%; this is the highest infection prevalence reported anywhere in Canada. Additionally, 43% of *I. scapularis* nymphs were infected with Lyme disease spirochetes; this is the highest nymphal infection rate for *I. scapularis* reported in Canada. Our findings show that people frequenting Corkscrew Island should take precautions to avoid contracting Lyme disease and associated tick-borne diseases.

Establishment on Corkscrew Island of *I. scapularis*. There are several possible ways that *B. burgdorferi* s.l.-infected *I. scapularis* could have become established on Corkscrew Island. Geographically, the closest point between the island and the mainland is 1.5 km (Figure 1). White-tailed deer are good swimmers, and can easily make the crossing; in fact, a Sitka black-tailed deer, *Odocoileus hemionus sitkensis*, was reported to have swum 22.5 km from one island to another island along Alaska's southeastern coast [32]. In late fall and spring, white-tailed deer have hollow hair, which adds buoyancy for long-distance crossings. When Lake of the Woods freezes in late December and early January for several months each winter, large mammals (i.e., white-tailed deer, black bear, gray wolves) can cross the ice from the mainland, unhindered. However, *I. scapularis* ticks are not questing in this frigid weather when sub-zero temperatures and snow cover prevail. Therefore, *I. scapularis* would not be introduced during the winter. With an overwinter survival of 93% at this site, we show that *I. scapularis* is well adapted to withstand cold climes. Black bears are also good swimmers, and have been seen swimming to Corkscrew Island. Black bears could, likewise, bring *I. scapularis* ticks to the island [33]. Additionally, a person with a companion animal, such as a dog, could introduce all 3 host-feeding life stages of *I. scapularis*. If a gravid female is introduced by a transient mammal from the mainland, it could oviposit in the leaf litter on Corkscrew Island; however, the progeny would not be infected with *B. burgdorferi* s.l. Transovarial transmission of *B. burgdorferi* s.l. is not present in *I. scapularis* ticks. Alternatively, a heavily-infested songbird with *I. scapularis* immatures could start an established population of *I. scapularis* [34]. Since songbirds transport *B. burgdorferi* s.l.-infected *I. scapularis* immatures, it is most likely avian hosts were the original mode of establishing a Lyme disease endemic area on Corkscrew Island.

A high prevalence of *B. burgdorferi* s.l. in an established population of *I. scapularis* indicates that Lyme disease spirochetes have likely been present for many years. For example, this phenomenon is borne out at Point Pelee National Park, Ontario, at the southern tip of Canada; there, Banerjee et al. [35] found that the *B. burgdorferi* s.l. infection prevalence in 1997 was nil. Later, Thorndyke [36] revealed that the prevalence of *B. burgdorferi* s.l. in *I. scapularis* adults shifted gradually and incrementally from 5.5% (2005) to 27.4 (2012). Although there was a fluctuation of *B. burgdorferi* s.l. presence from year to year, there was an increase in infection prevalence with time. Historically, Watson and Anderson [37] provide the first account of an established population of *I. scapularis* in Canada; field studies in 1972 and 1973 revealed all host-feeding life stages of *I. scapularis* at Long Point, Ontario. Because the infection prevalence (73%) of *B. burgdorferi* s.l. in *I. scapularis* adults on Corkscrew Island is higher than Long Point, Ontario (60%), we suggest that the *I. scapularis* breeding colony on Corkscrew Island pre-dates the one at Long Point, but was overlooked.

There is anecdotal evidence that patients have contracted Lyme disease on Corkscrew Island and the surrounding area. These patients developed multiple clinical symptoms indicative of Lyme disease, including progressive arthritis, neurological deficits, and profound fatigue. Of medical significance, Scrimenti [38] described an erythematous rash on a patient (a physician), who was bitten by a tick while grouse hunting in the fall of 1969 in Wisconsin; he represents the first recognized case of Lyme disease in North America in modern history. The attached tick was most likely an *I. scapularis* female because American dog ticks (*D. variabilis*) do not quest in October in this geographic area.

Based on accumulated degree-days and the placement of *I. scapularis* ticks in outdoor housing units, Lindsay et al. [39] postulated that the climate in the Kenora District, Ontario was not warm enough for *I. scapularis* to survive and become an established population. These researchers stated that *I. scapularis* would be limited to areas of Ontario south of an

imaginary line between North Bay and Thunder Bay, and westward to the Rainy River District, which is south of Kenora. Conversely, our study clearly shows that there are adequate degree-days for *I. scapularis* to thrive on Corkscrew Island. In the present study, blacklegged tick adults were winter hardy for 3 consecutive winters (2014, 2015, 2016). What was once considered by some researchers as a hostile environment for *I. scapularis* has turned out to be one of the most hyper endemic areas for Lyme disease in Canada.

Blacklegged ticks have an innate ability to withstand weather extremes [40]. Based on historical annual weather data, the maximum extreme high at Kenora was recorded at 36ºC, whereas the minimum extreme low was -44ºC. The normal accumulated snow cover is 22 cm (Environment Canada). Blacklegged ticks are adapted to these conditions because they have antifreeze-like compounds (glycoproteins) in their bodies [41]. Since sub-zero, ambient air temperatures prevail at Corkscrew Island throughout the winter, *I. scapularis* can survive in the leaf litter under an insulating blanket of snow. During hot summer days, they descend into the cool, moist leaf litter, and re-hydrate. Based on our studies, harsh ambient air temperatures are not a limiting factor in the survival of *I. scapularis* in the Kenora District.

High prevalence of *B. burgdorferi* s.l. On Corkscrew Island, there are several biotic factors that could contribute to the exceptionally high prevalence of *B. burgdorferi* s.l. in *I. scapularis*. Small mammals, which are reservoir-competent hosts for *B. burgdorferi* s.l. include: deer mice [42, 43], northern short-tailed shrew [44, 45], eastern chipmunks [46, 47], meadow voles [48], and southern red-backed voles [49]. Although white-tailed deer are incompetent reservoirs of *B. burgdorferi* s.l. [50], they act as amplifying hosts of *I. scapularis* ticks, and support their reproduction. Alternate hosts for *I. scapularis* adults include: woodchuck, American red squirrel, raccoon, red fox, gray wolf, and American black bear [51, 52]. Blacklegged tick males and females commonly mate on deer and, when females become fully engorged, they drop from their hosts into the leaf litter of tick-conducive habitats. Because blacklegged ticks are subject to desiccation, they favour sheltered woodlands and shady ecotones, and employ ambush strategies to parasitize their hosts.

When small mammals transect the microhabitat where *I. scapularis* females have deposited their eggs, they can become highly parasitized by hundreds of host-seeking larvae [34]. If these small mammals are already spirochetemic, they can transmit *B. burgdorferi* s.l. to the larvae during feeding and, subsequently, these replete larvae will transstadially pass Lyme disease spirochetes to nymphs during the larva-nymph moult. During the next blood meal, these nymphs can transmit spirochetes to the next hosts. Since white-footed mice, *Peromyscus leucopus*, are not present on Corkscrew Island, the high prevalence of *B. burgdorferi* s.l. in *I. scapularis* elucidates the fact that this small mammal is not needed to maintain a high level of borrelial endemicity. With such a high prevalence of *B. burgdorferi* s.l. in *I. scapularis* adults on Corkscrew Island, we found that the enzootic transmission cycle of *B. burgdorferi* s.l. is very efficient.

Questing activity of blacklegged ticks tied to oaks. In the present study, approximately 90% of the *I. scapularis* ticks were collected within 3 m of the trunks of bur oaks. Ostfeld et al. [53] found that whenever there is an abundant acorn crop, the number of mice significantly increased the following year and, likewise, the number of *I. scapularis* nymphs on white-footed mice strengthened. Large mast production provides highly nutritious food for both cricetid (i.e., deer mice) and sciurid (i.e., eastern chipmunks) rodents and white-tailed deer. Gravid females frequently drop from their hosts (i.e., white-tailed deer) in juxaposition to bur oaks. Stafford [54] discovered that *I. scapularis* larvae normally travel no more than 40 cm, but can crawl up to 2 m from the egg-laying site. In addition, Carroll [55] collected larvae on the trunks of oaks to a height of 2 m, which indicates that gravid females frequently drop from their hosts near oak trees. When we mapped the position of bur oaks and the sites where *I. scapularis* adults and nymphs were collected, we found that there was a direct correlation between these two biotic variables. Not only do bur oaks act as a source of high energy acorns, they provide a tick-conducive habitat for *I. scapularis*. As well, bur oaks act as a communal hub for deer and small mammals, and provide high-energy food for deer and reservoir-competent rodents. Moreover, other arboreal plants, such as American hazelnuts and Saskatoon berries, provide nutrition for rodents.

Blacklegged ticks use chemosensilla (sense organs) to detect ammonia, carbon dioxide, lactic acid, and various phenols [56]. These compounds play a vital role in finding their hosts. In particular, blacklegged ticks are attracted to host scent trails and the source of ammonia, which is generated by animal by-products (e.g., urine, faeces). Another tick attractant, lactic acid, is produced by mammalian hosts during normal metabolism and exercise. Phenols are present in urine, sweat, body odor, and estrogen hormones (i.e., estradiol), and are also released from decomposing leaf litter. Moreover, carbon dioxide from exhaled breath stimulates ticks,

and activates front leg flailing. Tick chemosensilla continue to be active as long as there is a chance of parasitizing an approaching host [56]. In the spring, gravid females commonly lay their eggs in the leaf litter in close proximity to bur oaks on Corkscrew Island, and start a new generation of *I. scapularis*.

When we flagged the leaf litter in the vicinity of bur oaks, we found that blacklegged tick nymphs were actively questing in late May through June. After nymphs parasitize a host and obtain a blood meal, they will moult to adults in 5 to 9 weeks. If they are not successful in parasitizing a host during the summer, they will overwinter and start host-seeking in the spring. Based on the presence of nut-producing oaks and highly-efficient, reservoir-competent hosts, Corkscrew Island has natural amenities (i.e., ideal microclimate, suitable hosts) to support an established population of *I. scapularis.* Moreover, the abundance of reservoir-competent hosts on Corkscrew Island helps to reinforce and sustain the enzootic transmission of *B. burgdorferi* s.l.

Presence of *I. scapularis* immatures on Corkscrew Island. In this study, we focused on the collection of *I. scapularis* adults because they are the easiest to collect and they have had two previous blood meals and represent the highest level of *B. burgdorferi* s.l. infectivity. Over the 3-year study period, we allowed enough time for this tick species to complete its entire life cycle. Rand et al. [57] found that when white-tailed deer, which are reservoir-incompetent hosts, were completely and permanently eliminated from Monhegan Island, 16 km off Maine's coast, the *B. burgdorferi* s.l. infection prevalence in *I. scapularis* adults dropped from 75% to 29% in four years. Based on their findings, we can hypothesize that *I. scapularis* larvae and nymphs are feeding on small mammals with a high prevalence of spirochete infection on Corkscrew Island, and that these ixodid immatures become infected with *B. burgdorferi* s.l. from spirochetemic hosts. Since unfed *I. scapularis* nymphs had infection prevalence of 43%, terrestrial small mammals are probably acting as the reservoirs for spirochetal infection. Because both *I. scapularis* nymphs and adults on Corkscrew Island have such an elevated prevalence of *B. burgdorferi* s.l., we have substantial evidence that Lyme disease spirochetes are cycling enzootically within this highly endemic focus.

Small mammals are maintenance hosts and birds are incidental hosts in the enzootic cycle of *B. burgdorferi* s.l. [44]. Without larvae and nymphs feeding on highly-infected *B. burgdorferi* s.l. reservoirs, *I. scapularis* adults would not be able to acquire high infectivity, namely 73%, in our study. Scott and Durden [21] found that bird-feeding *I. scapularis* nymphs collected in central and eastern Canada had an infection prevalence of 35%. Most significantly, when replete *B. burgdorferi* s.l.-infected *I. scapularis* nymphs drop to the leaf litter from avian hosts, they do not double their infection prevalence, and would not have obtained the infection prevalence of 73%. Since songbird-derived *I. scapularis* immatures only generate a *B. burgdorferi* s.l.-infection prevalence of 35% or less, we conclude that *I. scapularis* adults with an infection prevalence of 73% originate from terrestrial reservoir hosts on Corkscrew Island. In order for a high *B. burgdorferi* s.l. prevalence to be maintained, there must be large mammals for *I. scapularis* females to acquire blood meals, and males and females to mate. White-tailed deer, black bears, raccoons, red fox, and gray wolves act as suitable hosts on Corkscrew Island to facilitate mating of *I. scapularis* adults and propagate a new generation of *I. scapularis* ticks [33]. In addition, unfed nymphs are actively questing in late June for highly efficient, reservoir-competent, small- and medium-sized hosts on Corkscrew Island. With respect to spirochete infection, an unfed nymph is one and the same as a replete larva; the only difference, is that it has gone through the larva-nymph moult. Likewise, males and unfed females are analogous to fully engorged nymphs; only, they have advanced through the nymph-adult moult. With the collection of all 3 host-feeding life stages in a single year, we are assured that an established population of *I. scapularis* is present on Corkscrew Island. Moreover, our findings underpin the fact that this tick species is cycling through all life stages (egg, larva, nymph, adult). Now that we have studied *I. scapularis* for three years, and have allowed it to complete it's 2-year life cycle, we fulfil the criteria for an estabished population of *I. scapularis* on Corkscrew Island.

Transportation of *I. scapularis* to Corkscrew Island by songbirds. Migratory songbirds play a key role in the wide dispersal of *I. scapularis* larvae and nymphs. Peak northward songbird migration in Canada occurs during May and early June, and this time of year coincides with the peak questing period of *I. scapularis* nymphs. When Neotropical and southern-temperate passerines make landfall at food-rich stopovers located along their migration routes, they can be parasitized by *I. scapularis* larvae and nymphs. Along the flight path, tick-infested songbirds could release *I. scapularis* immatures on Corkscrew Island and the surrounding islands and on the mainland. Anderson & Magnarelli [44] reported 19 *I. scapularis* nymphs on an American Robin, *Turdus migratorius*, and 21 larvae on a Swamp Sparrow, *Melospiza georgiana*. If passerines are highly infested with *I. scapularis* immatures, they can initiate new foci

of *I. scapularis* [34]. These bird-feeding ticks can be infected with *B. burgdorferi* s.l. and other tick-associated pathogens. Passerines may also acquire *I. scapularis* immatures on Corkscrew Island and transport them to the surrounding islands and the mainland.

En route to the boreal forest, passerines widely disperse Lyme disease vector ticks across Canada during northbound spring migration [16-21, 58-63]. Long-distance migrants transport Neotropical ticks to Canada from as far south as Brazil [61-63]. Notably, Scott and Durden [21] found that 35% of the *I. scapularis* nymphs collected from songbirds in eastern and central Canada were infected with *B. burgdorferi* s.l. Since the infection prevalence in the *I. scapularis* adults on Corkscrew Island is double the level of infection in incoming replete, songbird-transported *I. scapularis* nymphs, we suggest that this tick population has a long history of being established for decades prior to 1972 in this northern locality.

Prevention strategies to minimize *I. scapularis*. Several attempts have been made in North America to minimize the presence of *I. scapularis*. When white-tailed deer were extirpated on Monhegan Island, Maine, the incidence of *I. scapularis* was reduced but not eliminated [57]; songbirds continue to introduce *I. scapularis* larvae and nymphs annually. On Corkscrew Island, birds can re-introduce *I. scapularis* immatures and, similarly, deer parasitized by *I. scapularis* adults, can swim to the island. Controlled burns have temporally reduced the number of *I. scapularis* ticks, but the tick population replenished itself within three years [64-66]. To survive, ticks hide in protective sites, such as topsoil cracks, earthworm holes, and rotten logs. In order to make the environment less conducive to ticks, seasonal cottage owners on Corkscrew Island should keep grass cut and leaves raked [67]. Timely acaricide sprays have helped to reduce the occurrence of *I. scapularis*, but have failed to completely eliminate *I. scapularis* colonies [68]. On Corkscrew Island, bur oaks should be cut down around cottages and outbuildings to deter deer and rodents. Compost bins exacerbate the tick problem because they attract rodents infested with ticks. At the end of the day, cottagers and visitors should do a full body tick check. If a tick is found attached, take a close-up, digital, colored photograph to document the tick bite. The attached tick should be removed promptly with fine-pointed stainless steel tweezers. Grip the hypostome (barbed mouthpart) at the surface of the skin, and gently and firmly pull tick straight out. The tick should be kept for identification and PCR testing. The tick can be preserved in a tightly sealed vial of rubbing alcohol or ethanol.

Human and zoonotic health considerations. Lyme disease is a zoonotic spirochetosis that is typically transmitted to humans and other vertebrates by ixodid ticks. Transmission normally occurs 24-48 hours after tick attachment [68]; however, Cook [69] reports transmission of Lyme disease spirochetes in less than 16 hours, especially if the tick salivary glands are infected. Notably, other tick-borne pathogens can be transmitted much quicker. For instance, Powassan virus can be transmitted in less than 15 minutes [70]. After transmission, Lyme disease spirochetes progress and circulate throughout the body, and can simultaneously affect many organs and tissues. Patients may have an erythematous rash (i.e., bull's-eye, homogenous, atypical, erythema multiforme); however, 42% or less, have a rash [71-74]. As this multisystem disease advances, patients can present with a diverse array of symptoms, including fatigue, flu-like symptoms, arthritis, inflammation, radicular pain, peripheral neuropathy, and cognitive dysfunction [75]. Spirochetes evade host defenses, locate intracellularly, and form more resistant forms [76]; they also attach to, invade, and kill B and T lymphocytes [77]. As the zoonosis advances, spirochetes produce neurotoxins that induce inflammatory cytokines (i.e., interleukin 1, interleukin 6, TNF-alpha) [78, 79], and can result in mitochondrial dysfunction, oxidative stress, and physical and hormonal abnormalities [79, 80]. If left untreated or inadequately treated, *B. burgdorferi* s.s. will sequester and persist in deep-seated tissue, including brain [81-83], bone [84], collagenous tissues (ligaments, tendons) [85, 86], eye [87], glial and neuronal cells [88, 89], muscle [90], and fibroblasts/scar tissue [91]. Since *B. burgdorferi* s.s. is pleomorphic, treatment must take into account diverse forms (i.e., spirochetes, round bodies, blebs, granules); collectively, they form slime-coated, polysaccharide matrices, called biofilms [92]. Persister cells, which survive antimicrobials, must be recognized in refractory cases [93]. Lyme disease, which often manifests as a chronic infection, can sometimes be fatal [71, 81, 94]. Since spirochetes lodge in human testicles, semen, and vaginal secretions, *B. burgdorferi* s.s. can be sexually transmitted [95, 96]. Early treatment is very important; delayed treatment of Lyme disease may be long and difficult [97, 98].

In conclusion, we collected all 3 host-feeding life stages of *I. scapularis*, and provide the first authentic report of an established population on Corkscrew Island, Kenora District. We document the northernmost known breeding colony of *I. scapularis* in Ontario. This northerly hyperendemic area for Lyme disease has a *B. burgdorferi* s.l. infection prevalence of 73%, and constitutes the highest known infection prevalence for *B. burgdorferi* s.l. in all of

Canada. Our study reveals that white-footed mice are not the primary reservoirs of *B. burgdorferi* s.l. at this site or possibly at other sites in North America. Not only is there a well-established population of *I. scapularis* on Corkscrew Island, ticks are infected with *B. burgdorferi* s.s., which is pathogenic to humans and certain domestic animals. Health-care providers need to be aware that anyone visiting Corkscrew Island during the temperate months can contract Lyme disease. Public health officials are legally obligated to warn the public that this Lyme disease hotspot poses a major public health risk.

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Competing Interests

The authors have declared that no competing interest exists.

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