

Lyme Disease Bacterium, *Borrelia burgdorferi* Sensu Lato, Detected in Multiple Tick Species at Kenora, Ontario, Canada

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Abstract

We detected the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.), in 8 species of ixodid ticks (Acari: Ixodidae) collected from mammalian hosts, including humans, at Kenora, Ontario, Canada. These 8 tick species include *Ixodes angustus*, *Ixodes banksi*, *Ixodes cookei* (groundhog tick), *Ixodes gregsoni*, *Ixodes muris* (mouse tick), *Ixodes scapularis* (blacklegged tick), *Haemaphysalis leporispalustris* (rabbit tick), and *Dermacentor albipictus* (winter tick). Based on PCR amplification, 39 (41%) of 94 ticks tested were positive for *B. burgdorferi* s.l. DNA sequencing of the flagellin B (*flaB*) gene of *B. burgdorferi* s.l. complex revealed the presence of *B. burgdorferi* sensu stricto (s.s.), which is pathogenic to humans, and causes diverse neurological manifestations in patients. Notably, we provide the first record of *B. burgdorferi* s.l. in *I. gregsoni*, and reveal a new distribution record for this tick in eastern and central Canada by extending the known range westward by 200 km. Our findings indicate that there may be a wide-ranging enzootic transmission cycle of *B. burgdorferi* s.l. within the ecosystem throughout the Kenora area. The health-care profession must be fully cognisant that Lyme disease is present in the Kenora area, and is a public health risk.

Keywords: Ticks; Mammalian hosts; Lyme disease; *Borrelia burgdorferi* sensu lato; Kenora; Ontario; Epidemiology; Public health

Abbreviations: PCR: Polymerase Chain Reaction; CNS: Central Nervous System

Introduction

The Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.), is typically transmitted to mammalian hosts, including humans, by ixodid (hard-bodied) ticks (Acari: Ixodidae) [1]. Each tick species has its own set of suitable hosts, and Lyme disease, a zoonosis, is incidental to humans and domestic animals [2,3]. Left untreated or inadequately treated, Lyme disease can inflict a myriad of multisystemic clinical manifestations.

The first isolation of *B. burgdorferi* s.l. in mainland Ontario [4] was from a blacklegged tick, *Ixodes scapularis* (northern populations formerly considered as *Ixodes dammini*), collected from a dog with no history of travel that was living in Kenora, Ontario. A 10-year tick-host study (1993-2002), which was conducted across Ontario, revealed several *B. burgdorferi* s.l.-infected *I. scapularis* ticks on domestic animals and humans in the Kenora District [5]. Recently, Scott et al. [6] documented an established population of *I. scapularis* on Corkscrew Island, which is 20 km southwest of Kenora. This hyperendemic

Lyme disease area has the highest infection prevalence of *B. burgdorferi* s.l. ever reported in Canada. It is evident that the climate in the Kenora area provides an environment that is conducive for *I. scapularis* reproduction.

Across North America, several *Ixodes* species are vector competent for *B. burgdorferi* s.l., including *I. affinis*, *I. angustus*, *I. dentatus*, *I. jellisoni*, *I. minor*, *I. muris*, *I. scapularis*, and *I. spinipalpis* [7]. Of these ticks, immature stages of *I. affinis*, *I. dentatus*, *I. minor*, *I. muris*, *I. scapularis*, and *I. spinipalpis*, and all host-seeking stages of *I. muris* can parasitize birds, and can be transported long distances into Canada during northward spring migration [8-17]. Each of these ixodid ticks has the ability to acquire spirochetes while feeding on an infected host and, during the moult, pass them transstadially to the next life stage.

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Subsequently, Lyme disease spirochetes can be transmitted to vertebrate hosts, including humans.

The aim of this study was to identify species of ticks in the Kenora District that may be involved in the enzootic cycle of *B. burgdorferi* s.l.

Materials and Methods

Study area

Ticks were collected from humans, domestic and wildlife animals within 50 km of Kenora, Ontario, Canada (49° 46' N, 94° 29' W) on the northern edge of Lake of the Woods. This geographic area lies within the Canadian shield, which is comprised of Precambrian igneous rock. The coniferous-deciduous forest lies within the boreal forest region, and consists primarily of: red pine, *Pinus resinosa*; eastern white pine, *Pinus strobus*; black spruce, *Picea mariana*; white spruce, *Picea glauca*; white birch, *Betula papyrifera*; trembling aspen, *Populus tremuloides*; bur oak, *Quercus macrocarpa*; and Manitoba maple, *Acer negundo*.

Tick collection

Ticks were collected from injured wild mammals, domestic animals, and from local residents, and submitted to local wildlife rehabilitators, wildlife biologists, and veterinarians. These ticks were removed using fine-pointed, stainless steel tweezers. Live ticks were placed in round-bottom, 8.5 mL polypropylene tubes (15.7 mm × 75 mm) (Sarstedt) with attached labels specifying host, date collected, geographic location, and collector's name. A 7-mm hole in the polyethylene cap (15.7 mm) provided ventilation for ticks and, to prevent the ticks from escaping, fine tulle netting was stretched over the open end of the tube before the push-cap was inserted. The tube, which contained the ticks from a single host, was placed in a double-zippered plastic bag to maintain high humidity.

Dead and damaged ticks were put in 2 mL micro tubes containing 94% ethyl alcohol. Both live and dead ticks were sent to the tick identification lab (JDS).

Fully engorged immature ticks were held to molt to the next developmental life stage. These engorged ticks were placed in 8.5 mL polypropylene tubes with a slightly moistened piece (5 cm × 5 cm) of chlorine-free paper towel. The open end of the tube was covered with tulle netting before the open-centered push cap was inserted. The tubes were placed in a double-zipper plastic bag with a slightly moistened paper towel to provide adequate humidity (~95%). A full spectrum, 12W LED, LifeLite light bulb (LifeEnergy Systems), on a timer, was set for a summertime photoperiod (16:8/L:D). Each plastic bag, which contained replete ticks, was checked every 3-5 days to assess the progress of the moult and to ensure that sufficient humidity was maintained. A log sheet was kept for each tick to record the dates checked, advancement of the moult, and the number of days to complete the moult.

Spirochete detection

Field-collected ticks were sent to 3 different laboratories for *B. burgdorferi* s.l. testing because the first two laboratories had policy changes during the study, and stopped testing ticks. The 3 laboratory periods are: May 2013–June 2013 (JFA), July 2014–June 2015 (KLC), and July 2015–November 2016 (JEF). At lab A (JFA), live ticks from mammalian hosts were cultured in Barbour-Stoenner-Kelly (BSK) medium, and dead ticks were tested directly using DNA extraction and PCR testing. The DNA detection protocols have been described previously [18-20]. Although Persing et al. [18] employed both the flagellin (*fla*) gene and the major outer surface protein (*OspA*) gene, lab A only used the *OspA* gene. Appropriate negative and positive controls were used.

In the second part of the study, ticks were placed in 94%

Tick species	Host species (no.)	No. of hosts	No. of ticks	Life stages of ticks				No. ticks Bbsl-pos./ No. of ticks tested (%)
				L	N	M	F	
<i>I. angustus</i>	dm (1)	1	1	0	0	0	1	1/1 (100)
<i>I. banksi</i>	bv (8)	8	11	0	8	0	3	6/11 (55)
<i>I. cookei</i>	gh (2), hm (1), mk (1)	4	17	0	12	0	5	6/17 (35)
<i>I. gregsoni</i>	er (2), mk (1)	3	17	0	14	0	3	4/12 (33)
<i>I. muris</i>	ct (2), dm (2), dg (5), mv (2), nss (2), srv (3)	16	16	2	8	0	6	7/16 (44)
<i>I. scapularis</i>	dg (9), hm (3)	12	15	0	0	2	13	5/15 (33)
<i>D. albipictus</i>	dg (2), ssh (1)	3	5	0	5	0	0	1/4 (25)
<i>H. leporispalustris</i>	ssh (5)	5	29	4	21	1	3	9/19 (47)
Total	12	52	111	6	68	3	34	39/94 (41)

Table 1: Detection of *B. burgdorferi* s.l. in ixodid ticks collected from mammalian hosts in the vicinity of Kenora, Ontario, 2013-2016. L, larva (e); N, nymph (s); M, male (s); F, female (s); Bbsl, *Borrelia burgdorferi* s.l.; dm, deer mouse; bv, beaver; gh, groundhog; hm, human; mk, mink; er, ermine; ct, cat; dg, dog; mv, meadow vole; nss, northern short-tailed shrew; srv, southern red-backed vole; ssh, snowshoe hare.

ethyl alcohol and forwarded to lab B (KLC). These ticks were PCR tested using two sets of primers for the flagellin B (*flaB*) gene and one for the 16S-23S rRNA intergenic spacer gene. The *flaB* PCR assays are species-specific for *B. burgdorferi* s.l., while the 16S-23S spacer PCR is *Borrelia* genus specific. The 16S-23S spacer PCR assay was used to confirm any of the positives obtained using the *flaB* primers. For ticks collected in the latter stage of the second part of the study, we only used the *flaB* gene PCRs. The methodology was described earlier in Scott et al. [6]. The negative control consisted of nuclease-free TE buffer. In order to prevent DNA contamination, a positive control was not used. Using the PCR1 and PCR2 primer sets, amplicons (194-bp, base position 313 to 506; 206-bp, base position 532 to 737) of the *B. burgdorferi* s.l. *flaB* gene were obtained from 2 ticks (*I. angustus*, 14-5A169; *I. scapularis*, 14-5A207).

In the latter part of the study, ticks were stored in 2 mL micro tubes containing 94% ethyl alcohol, and sent directly by courier to lab C (JEF) for testing; the protocols are outlined in Scott and Foley [21].

Because of the special significance of *I. gregsoni* in the present study, two ticks were kept as voucher specimens rather than being tested for *B. burgdorferi* s.l. These nymphal ticks (13-5A172, 16-5A96A) have been archived in 95% ethyl alcohol in the Centre of Biodiversity Genomics (CBG), University of Guelph with accession numbers BIO-15-068 and BIO-16-147, respectively. The DNA extracts are held at -80°C at the same location. DNA extraction, amplification, and sequencing followed the protocol previously described in Scott et al. [22]. The collection data and barcode sequences are stored on the Barcode of Life Datasystems (BOLD; www.boldsystems.org), and can be accessed in the BOLD dataset at: dx.doi.org/10.5883/DS-IGAK. In addition, the sequences have been deposited in GenBank with the accession numbers: KY370928 (for tick 13-5A172N) and KY370927 (for tick 16-5A96A).

The 194-bp nucleotide sequences of the *flaB* gene obtained from *B. burgdorferi* s.l. amplicons were deposited in GenBank with accession numbers: KT827327.1 (from an *I. angustus* female, 14-5A169) and KT807497.1 (from an *I. scapularis* female, 14-5A207).

Results

Tick collection

We obtained 111 ixodid ticks from 52 small-, medium-, and large-sized vertebrate hosts, including humans,

representing 12 different mammalian species in the Kenora area (Table 1). They include: cats, *Felis catus*; dogs, *Canis lupus familiaris*; deer mice, *Peromyscus maniculatus*; meadow voles, *Microtus pennsylvanicus*; northern short-tailed shrew, *Blarina brevicauda*; and southern red-backed voles, *Myodes gapperi*. In addition, we collected 20 American dog ticks, *Dermacentor variabilis*, from mammalian hosts (i.e., dog, deer mice, red fox); however, because this tick species lacks vector competence for *B. burgdorferi* s.l., we did not test any of these specimens.

Spirochete detection

Lyme disease spirochetes were detected in 8 different species of ticks (Table 1). Overall, 39 (41%) of 94 ticks tested were positive for *B. burgdorferi* s.l. When we conducted DNA sequencing with the *flaB* gene, we detected *B. burgdorferi* s.s. in an *I. angustus* female, 14-5A169 and likewise, in an *I. scapularis* female, 14-5A207.

Discussion

This study reveals that *B. burgdorferi* s.l. is widely disseminated within an interconnected wildlife ecosystem in the Kenora area. We found 8 different species of ticks in the Kenora area that were positive for *B. burgdorferi* s.l. These findings show that Lyme disease spirochetes are present in a wide diversity of ticks and mammals throughout this area. Overall, *B. burgdorferi* s.l.-infected ticks were collected from 12 different mammalian species, including humans.

The Kenora bioregion comprises a diverse and intricately-connected ecosystem that supports numerous ticks, birds, and mammals. This biodiverse system sustains at least 9 tick species. Several small mammals inhabiting the Kenora area are known to be reservoir-competent hosts of *B. burgdorferi* s.l., namely deer mice [23,24], eastern chipmunk, *Tamias striatus* [25,26], meadow voles [27], northern short-tailed shrew [4,28], and southern red-backed voles [29]. Although white-tailed deer are not competent reservoirs of *B. burgdorferi* s.l., they support the reproduction of *I. scapularis* ticks [30]. Although white-footed mice, *Peromyscus leucopus*, are not indigenous in the Kenora area, these cricetid rodents will retain *B. burgdorferi* s.l. spirochetemias for life [31,32].

In addition to mammals, resident and migratory songbirds disperse bird-feeding ticks in the area, especially during northward spring migration [8-17]. Not only do multiple host species support and maintain Lyme disease vector ticks, Neotropical and southern-temperate songbirds annually transport bird-feeding ticks (i.e.,

Ixodes affinis, *Ixodes minor*) into central Canada during spring migration from as far south as Brazil [10,11,15]. Not only are bird-feeding ticks transported long distances, resident birds disperse Lyme disease vector ticks during the nesting and post-fledgling periods during the summer. Collectively, avian and mammalian hosts are vertebrate cohabitants, and enhance the complex epidemiological cycle of *B. burgdorferi* s.l. in the Kenora area.

Not only is *I. scapularis* is a competent vector of *B. burgdorferi* s.l. [1,33], *I. angustus* has vector competency for *B. burgdorferi* s.l. [34], In addition, *Ixodes muris* is a competent vector and supports enzootic maintenance of *B. burgdorferi* s.l. [35]. These particular tick species, plus reservoir hosts, provide multiple opportunities for the interchange of *B. burgdorferi* s.l. These vector-host-pathogen associations help to maintain and propagate the presence of Lyme disease spirochetes within the environment in the Kenora area.

Ixodes angustus

A partially engorged *I. angustus* female was collected from a deer mouse near Kenora on 1 August 2014; this is the first report of a *B. burgdorferi* s.l.-infected *I. angustus* in Ontario (Figure 1A). Notably, *I. angustus* is a competent vector of Lyme disease spirochetes [34], and the deer mouse is a reservoir-competent host [23,24]. This nidicolous tick species feeds primarily on rodents and shrews, and the male stays in the host nest to mate with females [36].

Zoogeographically, *I. angustus* has a wide range across the Holarctic Region (including the Temperate Zone of North America) [36,37]. In Canada, Lyme disease spirochetes were initially isolated from *I. angustus* ticks in British Columbia [38]. In Washington State, Damrow et al. [39] described a human case of Lyme disease with an erythematous lesion, and intimated that the attached *I. angustus* female was likely the vector of *B. burgdorferi* s.l. There is further evidence to indicate that *I. angustus* acts as a bridge vector of *B. burgdorferi* s.l. to humans [7]. In the present study, DNA sequencing revealed that the *Borrelia* amplicon from the *I. angustus* female was *B. burgdorferi* s.s., which is pathogenic to humans and certain domestic animals.

Ixodes banksi

A partially engorged *I. banksi* nymph was collected from a beaver, *Castor canadensis*, on 15 May 2013 at Silver Lake North, Ontario. This constitutes the first report of a *B. burgdorferi* s.l.-infected *I. banksi* in northwestern Ontario. Previously, a *B. burgdorferi* s.l.-positive *I. banksi* was collected at Sault Ste. Marie, Ontario [40]. During 2013, ten *I. banksi* ticks (2 females, 8 nymphs) were collected from 7 beavers north of Kenora, Ontario. With one exception, all beavers were parasitized by at least one *B. burgdorferi* s.l.-infected *I. banksi*. A partially engorged *I. banksi* female, 16-5A99, was collected from a beaver on 15 November 2016 at Jim Lake (50.10N, 94.40W) near Redditt, Ontario (Figure 1B); this constitutes the northernmost collection of *I. banksi* in North America.

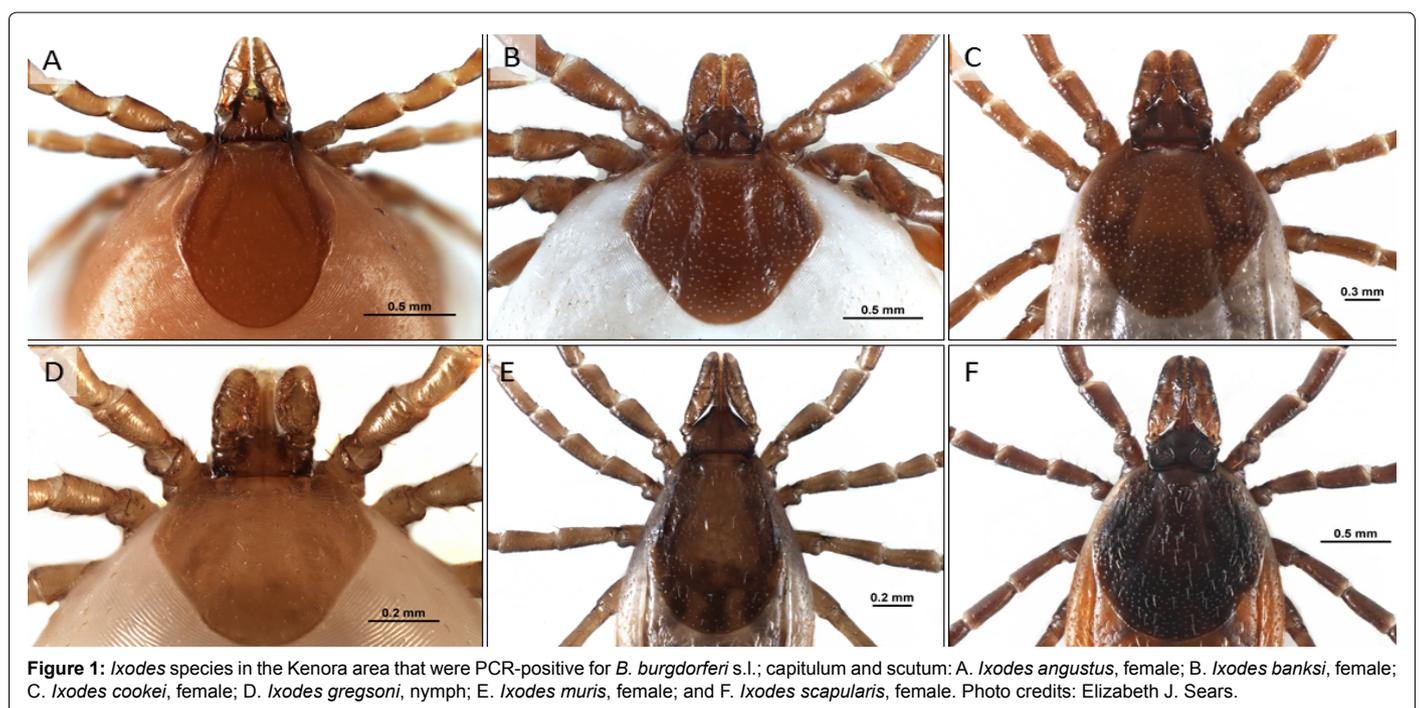


Figure 1: *Ixodes* species in the Kenora area that were PCR-positive for *B. burgdorferi* s.l.; capitulum and scutum: A. *Ixodes angustus*, female; B. *Ixodes banksi*, female; C. *Ixodes cookei*, female; D. *Ixodes gregsoni*, nymph; E. *Ixodes muris*, female; and F. *Ixodes scapularis*, female. Photo credits: Elizabeth J. Sears.

Of note, *I. banksi* is normally found on beavers and muskrats, *Ondatra zibethicus*, but it has also been recorded to parasitize humans [36]. Based on the nesting habits of beavers and muskrats, *I. banksi* is likely a nidicolous tick.

Ixodes cookei

The groundhog tick has a wide host range of terrestrial mammals, and has not been noted as a vector of Lyme disease spirochetes. Barker et al. [41] did not detect *B. burgdorferi* s.l. in any of the host-feeding life stages of *I. cookei* collected from humans and other animals, and purported that *I. cookei* is a poor vector of Lyme disease spirochetes. On the other hand, Hall et al. [42] reported a woman who had been bitten by an *I. cookei* nymph in West Virginia, and developed a typical erythema migrans (EM) rash. These latter researchers suggest that *I. cookei* is a vector of Lyme disease spirochetes. In the present study, 3 (60%) of 5 *I. cookei* on two groundhogs were infected with *B. burgdorferi* s.l. Notably, a human bitten by an *I. cookei* nymph developed symptoms typical of Lyme disease. In addition, a woman living in southwestern Ontario was bitten by an *I. cookei* nymph; this tick tested positive for *B. burgdorferi* s.l., and the woman developed classic symptoms associated with Lyme disease [43]. Our study provides strong circumstantial evidence that *I. cookei* is a vector of *B. burgdorferi* s.l., and that groundhogs are potential reservoirs of Lyme disease spirochetes.

Ixodes gregsoni

We provide the first record of *Ixodes gregsoni* positive for *B. burgdorferi* s.l. Twelve *I. gregsoni* ticks (1 female, 11 nymphs; 13-5A172) were collected from an ermine, *Mustela erminea*, near Kenora on 2 December 2013. Of the 8 nymphs tested, 4 were positive for *B. burgdorferi* s.l. One nymph was deposited in the tick collection of Lance A. Durden with accession number L3659. Likewise, one *I. gregsoni* nymph was deposited at the Centre for Biodiversity Genomics with accession number BIO-15-068; the Process ID is: TJSD015. Currently, this nymph represents the first *I. gregsoni* specimen with an associated sequence in GenBank. In addition, two *I. gregsoni* females were collected 45 km north of Kenora from an ermine on 15 November 2014; both females were negative for *B. burgdorferi* s.l. Previously, Lindquist et al. [44] reported *I. gregsoni* on mustelids as far west as Ignace, Ontario (49° 25' N, 91° 41' W).

We collected 3 *I. gregsoni* nymphs (16-5A96A, B, C) from an American mink, *Neovision vison*, at Jim Lake, near Redditt, Ontario on 5 November 2016 (Figure 1D). One of these *I. gregsoni* nymphs was retained for DNA

barcoding, and was deposited in the Centre for Biodiversity Genomics; this represents the second sequence record for this tick species. Our collections indicate that the range of *I. gregsoni* extends westward at least to Kenora and northward to Redditt, which is 200 km further west than Ignace, Ontario. This recent collection of *I. gregsoni* at Jim Lake is the northernmost collection of this tick species in North America. Our findings suggest that *I. gregsoni* ticks may have vector competency for *B. burgdorferi* s.l.; however, further transmission experiments would be required to determine its vector status.

Ixodes muris

We collected *I. muris* from 6 different mammalian host species, namely cats, dogs, deer mice, northern short-tailed shrews, meadow voles, and southern red-backed voles (Table 1). In all, 16 *I. muris* (6 females, 8 nymphs, 2 larvae) were collected from these mammals, and 7 (44%) of 16 engorged ticks were positive for *B. burgdorferi* s.l. (Table 1, Figure 1E). In nature, *I. muris* has vector competence for *B. burgdorferi* s.l. [35,45] and, consequently, this tick species can acquire spirochetes while feeding on an infected host, pass them transstadially during the moult, and later transmit Lyme disease spirochetes to a susceptible host during engorgement. *Ixodes muris* sometimes parasitizes cats and dogs, and has also been recorded from humans in New York, Pennsylvania and Rhode Island [46]. *Ixodes muris* immatures also parasitize rats [36], and all host-feeding life stages feed on ground-frequenting birds [8-10,12,15,36]. In essence, *I. muris* plays a supportive role in the enzootic maintenance of Lyme disease spirochetes in the Kenora area.

Ixodes scapularis

Blacklegged ticks (Figure 1F) were collected from local dogs and humans with no history of travel. In all, 5 (33%) of 15 *I. scapularis* adults were positive for *B. burgdorferi* s.l. One of the *Borrelia* amplicons from a *B. burgdorferi* s.l.-infected *I. scapularis* female underwent DNA sequencing, and was characterized as *B. burgdorferi* s.s., which is pathogenic to humans and certain domestic animals. Historically, Banerjee et al. [4] isolated *B. burgdorferi* s.l. from an *I. scapularis* in mainland Ontario; this tick was collected from a dog residing in Kenora, Ontario that had no history of travel. Additionally, a 10-year, tick-host study (1993-2002), which was conducted across Ontario [5], reported *B. burgdorferi* s.l.-infected blacklegged ticks in the Kenora District. Recently, Scott et al. [6] documented an established population of blacklegged ticks on Corkscrew Island in Lake of the Woods. Notably, this island has an

infection prevalence of 73% for *B. burgdorferi* s.l. in *I. scapularis* adults, the highest known prevalence of Lyme disease spirochetes in Canada. These researchers also found that white-footed mice, which host *I. scapularis* larvae and nymphs in some parts of North America, do not need to be present to maintain an enzootic cycle of *B. burgdorferi* s.l.

Songbirds (Passeriformes) can transport *B. burgdorferi* s.l.-infected *I. scapularis* larvae and nymphs to and from this bioregion during bi-directional migration in spring and fall [8-17]. Peak northward songbird migration in Canada occurs during May and early June, which coincides with peak questing periods of *I. scapularis* nymphs [47]. When southern-temperate and Neotropical songbirds make landfall at migratory stopovers along their flight path, they can be parasitized by *I. scapularis* immatures, especially nymphs. These bird-feeding ticks subsequently detach, and are released across Canada. As a result, people do not have to visit to another endemic area to contract Lyme disease. In addition, Scott et al. [22] recently discovered that raptors are hosts of *I. scapularis* ticks.

Biogeographically, *I. scapularis* is a common vector of Lyme disease spirochetes east of the Rocky Mountains; however, in the Kenora District, other *Ixodes* species (*I. angustus*, *I. banksi*, *I. cookei*, *I. muris*) also bite humans. At least 5 species of ticks could transmit *B. burgdorferi* s.l. to humans in this region. The present study reinforces the fact that *I. scapularis* plays a significant role in the epidemiological transmission cycle of *B. burgdorferi* s.l. in the Kenora bioregion.

Dermacentor albipictus

We provide the first record of the winter tick, *Dermacentor albipictus*, testing positive for *B. burgdorferi* s.l. On 9 December 2013, three *D. albipictus* nymphs were collected from a dog in the Kenora, Ontario area with no history of travel. Of particular note, 1 (33%) of 3 fully engorged nymphs was positive for *B. burgdorferi* s.l. We did not test the host dog to determine whether it might be infected with *B. burgdorferi* s.l. Since moose, *Alces alces*, are common hosts of all 3 host-feeding life stages of *D. albipictus*, it is possible that Lyme disease spirochetes were obtained during a larval bloodmeal from a spirochetemic animal. We are not aware of any previous findings of *D. albipictus* being infected with *B. burgdorferi* s.l.

Haemaphysalis leporispalustris

Three partially engorged nymphs of the rabbit tick, *Haemaphysalis leporispalustris*, were collected from a

snowshoe hare, *Lepus americanus*, on 11 October 2013. This lagomorph was located just north of Kenora. Of significance, 2 (67%) of 3 nymphs were positive for *B. burgdorferi* s.l. This discovery provides the first record of a *B. burgdorferi* s.l.-positive *H. leporispalustris* in Ontario. Previously, Banerjee et al. [48] isolated *B. burgdorferi* s.l. from *H. leporispalustris* collected from a snowshoe hare from Grande Prairie, Alberta; their discovery represents the first isolation of *B. burgdorferi* s.l. from rabbit ticks in Canada. Notably, the first *B. burgdorferi* s.l.-positive *H. leporispalustris* was collected from a Swainson Thrush, *Catharus ustulatus*, at Long Point, Ontario [15]. As well, *B. burgdorferi* s.l. was isolated from *H. leporispalustris* in Texas [49].

Other Tick Species

It is noteworthy that *Ixodes dentatus* larvae and nymphs are transported by migratory songbirds in eastern and central Canada during northward spring migration. Each year, these migrants can drop them in the Kenora bioregion during northward spring migration [8-10,12,15]. Moreover, all host-feeding life stages of *I. dentatus* collected from eastern cottontail in New York yielded *B. burgdorferi* s.l. [50]. This *Borrelia* was later named *B. andersonii* [51].

Throughout north-central and northeastern North America, several tick species are sympatric with *I. scapularis*. Likewise, several tick species are cohabitants in the Kenora area, and infest the same hosts. For example, the American dog tick, previously given is prolific in the Kenora area, but this tick species is not a competent vector of *B. burgdorferi* s.l. However, it is a vector of other tick-borne pathogens, namely *Francisella tularensis* (causative agent of tularemia), *Rickettsia rickettsii* (agent of Rocky Mountain spotted fever), *Ehrlichia chaffeensis* (agent of human monocytic ehrlichiosis), *Ehrlichia ewingii* (agent of canine granulocytic ehrlichiosis), and some attached females can cause tick paralysis [52].

Neurological manifestations of *B. burgdorferi* s.s

We detected *B. burgdorferi* s.s., which is pathogenic to humans, in ticks collected in the Kenora area. This particular *B. burgdorferi* s.l. genospecies can cause a wide diversity of neurological symptoms, especially in the peripheral nerves and central nervous system. As this spirochetosis advances in the body, the meninges, cranial nerves and peripheral nerve roots may be affected. Neurological Lyme disease includes all forms of encephalitis, encephalomyelitis, meningoencephalitis, and radiculomyelitis [53]. Of note, chronic encephalomyelitis is defined as lasting longer

than 6 months. *Borrelia burgdorferi* s.s. infection in the CNS is difficult to recognize because only 14% of patients recalled a tick bite at the site of an EM rash [54]. Another problem is the variable incidence of the EM rash, which ranges from 27% to 70% in Lyme disease studies [55,56]. Patients often see several physicians before the diagnosis is established. Long-lasting and remittent pain may be so intense that patients have significant difficulties with sleep and cannot maintain their jobs. Other complaints include flu-like symptoms with myalgia; arthralgia without joint swelling; profound fatigue and general malaise; weight loss; nausea and vomiting; transient weakness; and prickly sensations in extremities [57,58].

Not only are motor and sensory systems affected by neurological Lyme disease, balance is often altered. Ataxia of legs and gait occur in up to 60% of patients with CNS involvement [59]. Like other encephalitic diseases, chronic CNS Lyme disease often presents with neuropsychiatric symptoms. Concentration problems, fatigue, and depression may precede the onset of dementia-like symptoms [60]. Symptoms of dementia include cognitive impairment, memory loss, disorientation and, also psychiatric disorders [56]. In one patient who was suffering from a mild organic brain disorder, Alzheimer's disease was diagnosed before the patient died [61]. At autopsy, *B. burgdorferi* s.l. was detected in and cultured from the brain tissue. Other researchers have cultured and detected *B. burgdorferi* s.l. in the brains of psychiatric and Alzheimer's disease patients at autopsy [62-67]. Persistence of *B. burgdorferi* s.l. is clearly documented in Lyme disease patients [68-70].

Brain magnetic resonance imaging often shows hyperintense white matter lesions in patients with advanced neurological Lyme disease [53]. These lesions may mimic demyelination that occur in multiple sclerosis [58]. Neurological Lyme disease can be fatal in neonates and infants [71,72]. A newborn whose mother had suffered from Lyme disease during early pregnancy died 23 hours after birth, and *B. burgdorferi* s.l. was demonstrated in the brain and liver by silver staining and immunochemistry [73]. In addition, Lyme disease is sometimes fatal in children and adults [74,75]. In late stage neurological Lyme disease, patients may become bedridden and confined to a wheelchair. This multisystem disease can also lead to optic nerve damage and cause blindness [69], and leave patients compromised and sometimes unable to pursue education or occupation.

Conclusions

Our findings disclose that *B. burgdorferi* s.l. is widely disseminated in 8 species of ticks inhabiting the environment encompassing Kenora, Ontario. When humans contract Lyme disease, they can experience multiple clinical manifestations, including significant neurological involvement. Ultimately, *B. burgdorferi* s.s.-infected ticks have an interconnecting link with humans and companion animals in the Kenora area. Transmission experiments need to be completed to determine if some of these ticks, such as *I. gregsoni*, are competent vectors of *B. burgdorferi* s.l. Notably, humans and companion animals engaging in outdoor activities are vulnerable to contracting Lyme disease and associated tick-borne diseases. The medical practitioners must be aware that Lyme disease vector ticks pose a public health risk in the Kenora area.

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