Lyme Disease Spirochete, *Borrelia burgdorferi* Endemic at Epicenter in Rondeau Provincial Park, Ontario

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ABSTRACT The Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmidt, Hyde, Steigerwalt, and Brenner was discovered in blacklegged ticks, *Ixodes scapularis* Say at Rondeau Provincial Park, Ontario, Canada. During this 2-yr study, spirochetes were found in *B. burgdorferi*-positive *I. scapularis* larvae attached to *B. burgdorferi*-infected white-footed mice, *Peromyscus leucopus* Rafinesque. Isolates of *B. burgdorferi* were cultured from blacklegged tick adults, and confirmed positive with polymerase chain reaction by targeting *Osp*A and *rrf* (5S)-*rrl* (23S) genes. These findings show an endemic area for *B. burgdorferi* within an established population of *I. scapularis* at Rondeau Provincial Park.

KEY WORDS Lyme disease, *Borrelia burgdorferi*, blacklegged tick, *Ixodes scapularis*, Rondeau Provincial Park, Ontario

LYME DISEASE IS A bacterial zoonosis caused by *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner that is transmitted to humans by certain ixodid ticks (Burgdorfer et al. 1982). In eastern and central Canada, the blacklegged tick, *Ixodes scapularis* Say (northern populations previously treated as *I. dammini* [deer tick]) (Oliver et al. 1993, Keirans et al. 1996) acts as a competent vector of the Lyme disease spirochete (Burgdorfer and Gage 1986, Piesman and Sinsky 1988, Sanders and Oliver 1995). The blacklegged tick also acts as a vector of the etiologic microorganisms that cause human granulocytic ehrlichiosis (Pancholi et al. 1995, des Vignes and Fish 1997), human babesiosis (Piesman et al. 1986, Mather et al. 1990), and deer tick virus (Telford et al. 1997, Ebel et al. 1999), which is a variant of Powassan virus (Kuno et al. 2001). Recently, *Bartonella henselae*, the agent of cat scratch disease, has also been detected in *I. scapularis* (Eskow et al. 2001).

Morshed et al. (2000) reported *B. burgdorferi* in blacklegged tick adults collected from Rondeau Provincial Park, Ontario. As well, immature (larvae, nymphs) *I. scapularis* were noted on white-footed mice, *Peromyscus leucopus* Rafinesque, which act as a competent reservoir for *B. burgdorferi* (Bosler et al. 1983, Anderson et al. 1985, Donahue et al. 1987). Elsewhere in Ontario, Barker et al. (1988) first reported *B. burgdorferi* in *I. scapularis* (reported as *I. dammini*) at Long Point, Ontario, an endemic area for Lyme disease. *Borrelia burgdorferi*-positive blacklegged ticks that were removed from dogs, which had no out-of-province travel, and were serologically positive for Lyme disease, have been documented across mainland Ontario (Banerjee et al. 1995, 1996, 2000).

The blacklegged tick coexists in Rondeau Provincial Park with the American dog tick, *Dermacentor variabilis* Say, which is often noted by the public during summer months. Both species of ticks have established populations in the park and are often found together, because they frequently share similar climate, hosts, and environments.

In this 2-yr study, we examined ticks and small mammals to determine the presence of *B. burgdorferi*, its endemicity, and whether this spirochete is cycling enzootically at an epicenter in the south part of the park.

Materials and Methods

Study Area. Rondeau Provincial Park (42° 17′ N, 81° 51′ W) is a 3,254-hectare peninsula on the north shore of Lake Erie, in Chatham-Kent, Ontario. This cuspate sandspit is interspersed with ridges and sloughs, and consists of Carolinian hardwood forest dominated by red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), and black oak (*Q. velutina* Lam). The peripheral area opens up to savanna in which various prairie grasses are grazed by white-tailed deer, *Odocoileus virginianus* Zimmerman.

Tick Collection. Blacklegged tick adults were collected by flagging in 13 general areas throughout the park using a flannel-covered, waterproof crib sheet

Field collections were conducted under a research permit issued by the Ontario Ministry of Natural Resources.

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Table 1. Immature ticks collected from small mammal hosts at Rondeau Provincial Park, Ontario, 1999–2000

	P.l.	*B.b.*	*T.s.*	*I. scapularis*		*D. variabilis*		Overall prevalence of ticks on small mammals (%)
				Larvae	Nymphs	Larvae	Nymphs	
Early summer 26–28 June 1999	23	1	1	1	1	69	36	18/23 (72.0)
Late summer 30 Aug–4 Sept 1999	52	0	3	51	0	22	9	42/55 (76.4)
Late summer 11–15 Aug 2000	17	0	1	146	0	18	5	17/18 (94.4)
Total	92	1	5	198	1	109	50	77/98 (78.6)

P.l., Peromyscus leucopus; B.b., Blarina brevicauda; T.s., Tamias striatus.

(Dundee Mills, New York, NY); the collections coincided with their bimodal activity at this site in the spring and fall. Males and unfed females were stored in separate vials, and kept in ziplock bags with moist paper towel. Live ticks were sent promptly by courier for culturing to the British Columbia Centre for Disease Control. Adult *D. variabilis* were observed but not collected. An ixodid specimen was sent to Georgia Southern University for identification, and then forwarded to the British Columbia Center for Disease Control.

Larval and nymphal ticks were collected from live-trapped small mammals during early and late summer using 52–59 live-traps per night. Fully engorged, immature ticks were kept live for culturing, and handled in a similar way to adults; however, nonengorged or partially engorged ticks were put directly into microvials containing 70% isopropyl alcohol for polymerase chain reaction (PCR) testing. In this study, ticks were tested in pools of up to five adults (normally 3) or 28 larvae.

Culturing of *B. burgdorferi* from Ticks. Live ticks were surface sterilized with 10% H_2O_2 for 10 min followed by 70% isopropyl alcohol, and washed three times with sterile distilled water. The midgut was removed and placed in BSK II media as described previously (Barbour 1984, Scott et al. 2001), incubated at 34°C, and checked weekly by dark-field microscopy for live spirochetes for up to 30 d.

Culture of *B. burgdorferi* from Organs. Small mammals were killed using carbon dioxide, and frozen promptly for transit to the laboratory where they were thawed, soaked in 70% isopropyl alcohol, and washed three times in sterile water. Ear, spleen, liver, lung, heart and urinary bladder tissues were placed in BSK II media as described previously (Barbour 1984), and incubated at 34°C for 30 d. Tubes were checked for live spirochetes weekly.

Polymerase Chain Reaction. DNA was extracted from pure or contaminated cultures using Qiagen tissue kits (QIAGEN, Mississauga, ON). PCR was performed to amplify a portion of the variable spacer region between two conserved structures, the 3′ end of the 5S rRNA (rrf) and the 5′ end of the 23S rRNA (rrl), as described previously (Postic et al. 1994), and, similarly, a portion of the *Osp*A gene (Persing et al. 1990).

The PCR mixture for the variable spacer region consisted of one commercial bead containing 1.5 U of *Taq* polymerase (Roche Diagnostics, Quebec, QC), 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate (dNTP) (Roche Diagnostics, Quebec, Canada) and stabilizers including bovine serum albumin (Amersham Pharmacia Biotech, Quebec, Canada), 1 μl (20 pmol) primer one (CTGCGAGTTCGCGGGAGA), and 1 μl (20 pmol) primer two (TCCTAGGCATTCACCATA), both from the same supplier (Sigma, Oakville, ON), and 10 μl extracted DNA in a total volume of 30 μl. Thermal cycling consisted of 5 min at 94°C, 50 cycles for 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C, and a final 7-min extension at 72°C.

The PCR mixture for the *Osp*A gene consisted of one commercial bead containing 1.5 U of *Taq* polymerase, 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP and stabilizers including bovine serum albumin, 1 μl (20 pmol) primer three (TTCTGACGATCTAGGTCAAA) and 1 μl (20 pmol) primer four (GCAGTTAAAGTTCCTTCAAG) and 10 μl extracted DNA in a total volume of 30 μl. Thermal cycling consisted of 5 min at 95°C, 50 cycles for 1.5 min at 95°C, 1 min at 55°C, and 1.83 min (110 s) at 72°C, and a final 7-min extension at 72°C.

Negative and positive controls were used for all PCR reactions. The negative control employed sterile water, and the positive control used purified *B. burgdorferi* strain B31. The amplification products were analyzed by electrophoresis in 2.0% agarose gels followed by staining with ethidium bromide and visualization in ultraviolet light.

Results

Tick and Small Mammal Collection. Over the 2-yr study (1 April 1999–30 November 2000) both *I. scapularis* and *D. variabilis* were obtained in the park. A total of 358 immature ticks (199 *I. scapularis*, 159 *D. variabilis*) were removed from 98 small mammals during three summer field trips consisting of 679 live-trap nights (Table 1). These larval and nymphal ticks were collected primarily from the south part of the park. With the exception of one *I. scapularis* larva on an eastern chipmunk, *Tamias striatus* (L.), larval and nymphal ticks were removed from white-footed mice. No ticks were found on the single northern short-tailed shrew, *Blarina brevicauda* (Say), which was caught. Overall, tick prevalence on small mammals was 78.6% (77/98). For *I. scapularis*, the tick prevalence on small mammals was 45.9% (45/98) with a mean intensity on infested hosts of 4.4 (range, 1–30).

A total of 263 blacklegged tick adults (125 males, 138 females) were collected by flagging. During 39.2 h of flagging, an average of 6.7 adults were collected per hour with the majority obtained in the South Point Trail area. Interestingly, one gravid *I. scapularis* female removed from a dog visiting the park laid 3066 eggs, which later developed into viable larvae.

Spirochete Detection. At the epicenter, *I. scapularis* larvae attached to six white-footed mice were PCR-positive for *B. burgdorferi*, and the corresponding hosts were also positive using PCR. In all six cases, both target genes were positive. We did not find any differences in sensitivity between the two primer sets (variable spacer region between 5S rRNA [rrf] and 23S rRNA [rrl]; *Osp*A gene). Consistently, the second primer set amplicons confirmed the first primer set amplification products. Notably, six (40.0%) of 15 white-footed mice from the epicenter area were infected with *B. burgdorferi*.

Based on all *I. scapularis* adults collected from the park, 12 (14.0%) of 86 pools were PCR-positive for *B. burgdorferi*. Specifically, of adults collected from the epicenter, 10 (33.3%) of 30 pools were positive. In the remainder of the park, two (3.6%) of 56 pools showed positivity. Live cultures were obtained from *I. scapularis* adults collected from the epicenter and elsewhere in the park. All immature *D. variabilis* removed from *B. burgdorferi*-infected white-footed mice were negative by PCR for the Lyme disease spirochete.

Discussion

Infected mice and ticks collected at several sites in Rondeau Provincial Park confirm that *B. burgdorferi* is present, especially at the epicenter. Although *B. burgdorferi* was not detected in the north part of the park, established populations of *I. scapularis* and *D. varibilis* are sympatric there. Even though *D. variabilis* is established in the park, and is often noted by the public, it is not a competent vector of the Lyme disease spirochete (Piesman and Sinsky 1988, Sanders and Oliver 1995, Johns et al. 2001).

All of the small mammals caught in the park are competent reservoirs of *B. burgdorferi:* white-footed mice (Bosler et al. 1983, Anderson et al. 1985, Donahue et al. 1987), northern short-tailed shrew (Telford et al. 1990), and eastern chipmunk (McLean et al. 1993). These three mammalian species act as hosts for larval and nymphal *I. scapularis*. In contrast, white-tailed deer, which are common in the park, are incompetent reservoirs of *B. burgdorferi* (Telford et al. 1988); however, they do act as an important host of all motile stages of *I. scapularis*, especially adults (Durden and Keirans 1996). Similarly, the raccoon, *Procyon lotor* (L.), common in the park, is an inefficient reservoir of the spirochete (Ouellette et al. 1993, Norris et al. 1996).

Morshed et al. (2000) provided the first direct evidence of an established population of *I. scapularis* at the park. Songbirds likely introduced immature *I. scapularis* during spring migration from the South. Scott et al. (2001) reported wide distribution of *I. scapularis* in Canada on passerine birds extending from northern Alberta to Nova Scotia, some of which are infected with *B. burgdorferi*. Notably, some species of birds act as competent reservoir hosts of *B. burgdorferi*, and provide an avenue to introduce infected larvae and nymphs (Anderson et al. 1986, 1990, Weisbrod and Johnson 1989, Stafford et al. 1995, Richter et al. 2000). After fully engorged larvae drop to the ground, they molt, and as nymphs may infect mice. The avian checklist for Rondeau Provincial Park area reports 343 species of birds, most of which overwinter in southern latitudes (personal communication: Allen Woodliffe).

In conclusion, we provide direct evidence that the blacklegged tick is established in the park with all three motile stages present. *Borrelia burgdorferi* was discovered in both immature and adult *I. scapularis* at the endemic area in the south part of the park. Moreover, *B. burgdorferi*-positive *I. scapularis* attached to *B. burgdorferi*-infected white-footed mice were collected at the focal area of the park.

Because blacklegged ticks have a marked increase in questing activity at temperatures ≥4°C (Duffy and Campbell 1994), visitors should take tick preventative measures and stay on groomed trails. Physicians must be aware that patients may encounter *B. burgdorferi*-infected *I. scapularis* in the park, and subsequently contract Lyme disease. The Lyme disease spirochete is endemic at Rondeau Provincial Park, as it is present in both immature and adult blacklegged ticks, and white-footed mice.

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