LYME DISEASE IS A BACTERIAL ZOONOSES CAUSED BY BORRELIA BURGDORFERI. JOHNSON, SCHMIDT, HYDE, STEIGERWALT, AND BRENNER WAS DISCOVERED IN BLACKEGGED 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 RufsCQUE. ISOLATES OF B. BURGDORFERI WERE CULTURED FROM BLACKEGGED TICK ADULTS, AND CONFIRMED POSITIVE WITH POLYMERASE CHAIN REACTION BY TARGETING OSP A AND rrf (SS)-Irf (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, BLACKEGGED TICK, IXODES SCAPULARIS, RONDEAU PROVINCIAL PARK, ONTARIO.

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 Roussan Provincial Park, Ontario, 1999–2000

<table>
<thead>
<tr>
<th>Pl.</th>
<th>B.B.</th>
<th>T.L.</th>
<th>L. scapularis</th>
<th>D. variabilis</th>
<th>Overall prevalence of ticks on small mammals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Larvae</td>
<td>Nymphs</td>
<td>Larvae Nymphs</td>
</tr>
<tr>
<td>Early summer 20–22 June 1999</td>
<td>22</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>65 36</td>
</tr>
<tr>
<td>Late summer 20- Aug 1 Sept 1999</td>
<td>72</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>92 9</td>
</tr>
<tr>
<td>Late summer 11-18 Aug 2000</td>
<td>17</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>18 5</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>169 56</td>
</tr>
</tbody>
</table>

P.I., Peromyscus leucopus; B.B., Blarina brevicauda; T.L., 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 zip lock 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 isodic specimen was sent to Georgia Southern University for identification, and then forwarded to the British Columbia Centre for Disease Control.

Larval and nymphal ticks were collected from live-trapped small mammals during early and late summer using 35–59 live-traps per night. Fully engorged, immature ticks were kept live for culturing, and handled in a similar way to adults; however, none engorged or partially engorged ticks were put directly into microwells 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 25 larvae.

Culturing of B. burgdorferi from Ticks. Live ticks were surface sterilized with 10% H2O2 for 10 min followed by 70% isopropyl alcohol, and washed three times with sterile distilled water. The nudget 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. Tissues 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 (rf) and the 3’ end of the 23S rRNA (rt), as described previously (Postic et al. 1984), and, similarly, a portion of the OsPA 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 MgCl2, 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 (GTGGCCAGT-TCGGCGGAGA)G, and 1 μl (20 pmol) primer two (TCTCCGCGATTCCAGATA)G, 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 OsPA 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 MgCl2, 200 μM of each dNTP and stabilizers including bovine serum albumin, 1 μl (20 pmol) primer three (TCTGGAGCTACGTCAAAG) and 1 μl (20 pmol) primer four (GCAGTTAAGCTTCCAGAAG) 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 min at 95°C, 1 min at 53°C, and 1.5 min at 110°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 L. scapularis and D. variabilis were obtained in the park. A total of 368 immature ticks (199 L. scapularis, 169 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 L. 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 L. scapularis, the tick prevalence on small mammals was 45.9% (45/98) with a mean intensity on infected hosts of 4.4 (range, 1–30).
A total of 363 blacklegged tick adults (125 males, 138 females) were collected by flagging. During 59.2 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 L. scapularis female was removed from a dog visiting the park laid 3066 eggs, which later developed into viable larvae.

Sporochete Detection. At the epicenter, L. 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 [ref] and 23S rRNA [ml]; Ospa 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 L. 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 L. 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. variabilis 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 (Fleming and Sinskey 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. 1999), and eastern chipmunk (McLean et al. 1993). These three mammalian species act as hosts for larval and nymphal L. scapularis. In contrast, white-tailed deer, which are common in the park, are incompetent reservoirs of B. burgdorferi (Telford et al. 1985); however, they do act as an important host of all mollicute stages of I. scapularis, especially adults (Durean and Keirans 1990). Similarly, the raccoon, Procyon lotor (L.), common in the park, is an inefficient reservoir of the spirochete (Ouellette et al. 1999, 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 345 species of birds, most of which overwinter in southern latitudes (personal communication: Allen Woodcliffe).

In conclusion, we provide direct evidence that the blacklegged tick is established in the park with all three mollicute 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 24°C (Duffy and Campbell 1984), 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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