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WIDESPREAD DISPERSAL OF *BORRELIA BURGDORFERI*–INFECTED TICKS COLLECTED FROM SONGBIRDS ACROSS CANADA

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ABSTRACT: Millions of Lyme disease vector ticks are dispersed annually by songbirds across Canada, but often overlooked as the source of infection. For clarity on vector distribution, we sampled 481 ticks (12 species and 3 undetermined ticks) from 211 songbirds (42 species/subspecies) nationwide. Using PCR, 52 (29.5%) of 176 *Ixodes* ticks tested were positive for the Lyme disease spirochete, *Borrelia burgdorferi* s.l. Immature blacklegged ticks, *Ixodes scapularis*, collected from infested songbirds had a *B. burgdorferi* infection prevalence of 36% (larvae, 48%; nymphs, 31%). Notably, *Ixodes affinis* is reported in Canada for the first time and, similarly, *Ixodes auritulus* for the initial time in the Yukon. Firsts for bird-parasitizing ticks include *I. scapularis* in Quebec and Saskatchewan. We provide the first records of 3 tick species cofeeding on passerines (song sparrow, Swainson's thrush). New host records reveal *I. scapularis* on the blackpoll warbler and Nashville warbler. We furnish the following first Canadian reports of *B. burgdorferi*–positive ticks: *I. scapularis* on chipping sparrow, house wren, indigo bunting; *I. auritulus* on Bewick's wren; and *I. spinipalpis* on a Bewick's wren and song sparrow. First records of *B. burgdorferi*–infected ticks on songbirds include the following: the rabbit-associated tick, *Ixodes dentatus*, in western Canada; *I. scapularis* in Quebec, Saskatchewan, northern New Brunswick, northern Ontario; and *Ixodes spinipalpis* (collected in British Columbia). The presence of *B. burgdorferi* in *Ixodes* larvae suggests reservoir competency in 9 passerines (Bewick's wren, common yellowthroat, dark-eyed junco, Oregon junco, red-winged blackbird, song sparrow, Swainson's thrush, swamp sparrow, and white-throated sparrow). We report transstadial transmission (larva to nymph) of *B. burgdorferi* in *I. auritulus*. Data suggest a possible 4-tick, i.e., *I. angustus*, *I. auritulus*, *I. pacificus*, and *I. spinipalpis*, enzootic cycle of *B. burgdorferi* on Vancouver Island, British Columbia. Our results suggest that songbirds infested with *B. burgdorferi*–infected ticks have the potential to start new tick populations endemic for Lyme disease. Because songbirds disperse *B. burgdorferi*–infected ticks outside their anticipated range, health-care providers are advised that people can contract Lyme disease locally without any history of travel.

Birds act as hosts of several species of hard ticks (Acari: Ixodidae) that may be infected with tick-borne, pathogenic microorganisms. East of the Rocky Mountains, the blacklegged tick, *Ixodes scapularis* Say, acts as the principal vector for the spirochetal bacterium *Borrelia burgdorferi* sensu lato (hereafter *B. burgdorferi*), the etiological pathogen of Lyme disease (Burgdorfer and Gage, 1986; Anderson, 1988; Sanders and Oliver, 1995). Collectively, at least 76 different passerines (Passeriformes) are known as avian hosts of immature (nymphs, larvae) *I. scapularis*. In far-western Northern America, the western blacklegged tick, *Ixodes pacificus* Cooley and Kohls, is ascribed as the primary vector of Lyme disease (Burgdorfer et al., 1985; Lane and Lavoie, 1988); this tick species has been recorded on 42 species of passerines (Castro and Wright, 2007). Lyme disease–transmitting ticks have been reported in each of the 10 Canadian provinces (Sperling and Sperling, 2009). Taxonomically, *B. burgdorferi* has been detected in 6 different passerine-feeding *Ixodes* species, i.e., *Ixodes affinis* Neumann, *Ixodes dentatus* Marx, *Ixodes minor* Neumann, *Ixodes muris* Bishopp and Smith, *Ixodes pacificus*, *Ixodes scapularis*, and *Ixodes spinipalpis* Hadwin and Nuttall in indigenous areas, and scattered to extralimital locations in Canada, the United States, and Mexico (Durden and Keirans, 1996; Eisen and Lane, 2002).

Globally, the *B. burgdorferi* complex consists of at least 18 genospecies, including the recently delineated *Borrelia bavariensis*, *Borrelia kurtenbachii*, and *Borrelia yangtze* (Margos et al., 2010), and most recently *Borrelia finlandensis* (Casjens et al., 2011). Borrelial studies reveal genotype variation and marked heterogeneity across the United States and Europe (Crowder et al., 2010). In Canada, 4 different bird-associated *Ixodes* species (*I. auritulus*, *I. muris*, *I. pacificus*, and *I. scapularis*) have been noted as being infected with different genotypes of *B. burgdorferi* (Scott et al., 2010), and, in Ontario, cluster analysis using DNA sequencing divulged 4 different clades of this pathogenic spirochete (Morshed et al., 2006). Significant genetic diversity of *B. burgdorferi* has been noted in the Long Point bioregion situated along the north shore of Lake Erie (Scott and Durden, 2009).

As aerial transporters, songbirds have the propensity for short- and long-distance dispersal of attached ticks (Anderson and Magnarelli, 1984; Scott et al., 2001; Reed et al., 2003; Hamer et al., 2011). During spring migration, 2.5–3 billion passerines transport millions of Lyme disease vector ticks northward into Canada (Ogden et al., 2008; Scott et al., 2010). Infested songbirds, which are parasitized with *B. burgdorferi*–infected ticks, are able to start new foci of breeding populations that become Lyme-endemic areas (Anderson et al., 1990). However, in clinicians' offices, health professionals often postulate that people must frequent an endemic area, namely, in the United States, Europe, or Asia or one along the southern fringe of Canada, to contract Lyme disease. The epidemiology of Lyme disease in nature appears to be much more disseminated than is currently appreciated in either human or veterinary medicine. As a result, patients may not receive accurate diagnosis and treatment and, subsequently, may develop chronic, persistent Lyme disease.

Canadian government officials often remind people to take precautions when visiting endemic areas. However, in Ontario, the province reporting the highest number of human Lyme disease cases (2007–2010), 61% of the locally acquired cases had no exposure at an endemic area (K. Hay, pers. comm.). In November 1988, Lyme disease became a reportable disease in Ontario, and visiting an endemic area has been part of the surveillance criteria (Anonymous 1991).

The aim of the present cross-Canada study was 4-fold: (1) report Lyme-carrying ticks at new geographic areas; (2) investigate previously unrecorded *Ixodes* tick species at key locations; (3) provide current *B. burgdorferi* infection prevalences for *Ixodes* ticks on songbirds; and (4) re-evaluate the assumption that patients must visit a known endemic area to contract Lyme disease.

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MATERIALS AND METHODS

Tick collection

Ticks were removed from field-caught songbirds mist-netted by bird banders, and birds injured after building collisions, window strikes, and predation by domestic cats. These ticks were placed in 2-ml micro-tubes containing a small piece of non-chlorinated paper towel. A single drop of water was inserted on the tissue before capping. Micro-tubes were labeled with background data, placed in a self-sealing plastic bag with slightly moistened paper towel, and sent by courier to the field laboratory. Ticks were recorded, and identified to stage, engorgement, and species (J.D.S.). After identification, ticks were sent to the Connecticut Agricultural Experimental Station for *B. burgdorferi* DNA testing (J.F.A.). Some ticks were discarded because they were spoiled, whereas uncommon ticks were sent to Georgia Southern University for confirmation of identification (L.A.D.). The 3 undetermined, novel ticks (Lyme Disease Association of Ontario accession numbers: 08-5A6, 08-5A14, 08-5A16) are being held by authors (J.D.S., L.A.D.) for further morphological and genetic studies. Fully engorged, immature ticks were held at the field laboratory to molt before being sent for polymerase chain reaction (PCR) testing. Rabbit ticks, *Haemaphysalis leporispalustris* Packard, were not tested, because they had previously tested negative for *B. burgdorferi* (Scott et al., 2001; Morshed et al., 2005). However, Brown (1945) reported *H. leporispalustris* attached to, and feeding on, a man handing and butchering rabbits. Although *H. leporispalustris* is not known to transmit *B. burgdorferi* to humans (Lane et al., 1991), it has been demonstrated to harbor Lyme infection (e.g., Lane and Burgdorfer, 1988; Banerjee et al., 1995). Since the PCR results of ticks collected in 2007 have already been cited (Scott and Durden, 2009), they are not reported here.

Spirochete detection

Each unengorged or partially fed tick was tested for the presence of *B. burgdorferi* using PCR by methods described by Persing, Telford, Spielman, and Barthold (1990) and Persing, Telford, Rys, et al. (1990). Whole ticks were ground with a large paper clip in a 0.6 ml microcentrifuge tube containing 25 ul to 35 ul K Buffer. A different paper clip was used for each tick. Each tick was then boiled at 94 C for 10 min. DNA was extracted from engorged ticks following instructions in the QIAamp DNA Mini Kit (250) (QIAGEN, Valencia, California). Primers were the *ospA* gene target: ospA2, 5′-GTTTTGTAATTTCAACTGCTGACC-3′; ospA4, 5′-CTGCAGCTTGGAATTCAGGCACTTC-3′. PCR amplification was performed using a Perkin-Elmer thermal cycler set at 94 C for 45 sec, 45 C for 45 sec, and 72 C for 1 min for a total of 45 cycles. Appropriate negative and positive controls were used. Amplification products were analyzed by electrophoresis, stained with ethidium bromide, and examined under UV illumination as described by Persing, Telford, Spielman, and Barthold (1990) and Persing, Telford, Rys, et al. (1990). Amplification products were transferred to a nylon membrane by Southern blot. The membrane was then hybridized overnight with ^{32}P using the probe ospA3, 5′-GCCATTTGAGTCGTATTGTTGTACTG-3′. The membrane was then washed, and Kodak X-OMAT AR film was placed over the membrane for 4 hr. Infected ticks were detected with the ^{32}P probe.

RESULTS

Tick collection

During this tick-host-pathogen study (2007–2009), which encompassed 21 sites from Canada's east coast to Vancouver Island, British Columbia (B.C.), we obtained a total of 481 ticks consisting of 12 ixodid tick species, plus 3 undetermined nymphal *Ixodes* ticks collected from 211 wild-caught passerines representing 42 bird species/subspecies (Fig. 1). Of all tick-infested birds, the Swainson's thrush was the bird species with the most ticks (Table I) and was also parasitized by the highest number of *B. burgdorferi*–positive ticks (Table II), followed closely by the song sparrow. A Swainson's thrush was infested with 52 *H. leporispalustris* (6 nymphs, 46 larvae) at Toronto, Ontario (Site 7) on 10 September 2007 and constitutes the highest number of ticks reported from an individual songbird in Canada. A nymphal *H. leporispalustris* engorging on a hermit thrush is illustrated in Figure 2.

In western Canada, we provide the first host record of *I. spinipalpis* on a songbird on Vancouver Island, B.C.; a nymph was collected from a song sparrow on 17 August 2008 at Maltby Lake, B.C (Site 16). On the central flyway, an *I. scapularis* nymph was collected from a Swainson's thrush on 30 May 2007 at Tofield, Alberta (Site 14). At a northern inland site, 2 *I. auritulus* larvae were documented on a Wilson's warbler on 11 May 2008 at Watson Lake, Yukon (Site 21). Three species of ticks (*I. affinis* [1 nymph, 3 larvae], 4 *I. dentatus* larvae [2 *B. burgdorferi*–positive], and 1 *I. scapularis* nymph [*B. burgdorferi*–positive]) were detached from a Swainson's thrush at Delta Marsh, Manitoba (Site 12) on 27 May 2008.

Extended distribution of Lyme disease vector ticks is evident during northward spring migration in Canada. Within the Atlantic flyway, a fully engorged *I. scapularis* nymph was collected from a white-throated sparrow on 14 May 2007 on Bon Portage Island, Nova Scotia (Site 2). Similarly, 2 fully engorged *I. scapularis* larvae were collected from a northern waterthrush on 25 May 2007. Because these bird-feeding ticks had engorged on Neotropical songbirds for 4–5 days during northward migratory flight from the U.S. eastern seaboard, these *I. scapularis* collections provide further evidence of cross-border (U.S.–Canada) tick movement. In Ontario, an *I. scapularis* nymph was removed from a blackpoll warbler on 31 May 2007 at Toronto (Site 7) and a nymphal *I. scapularis* was removed from a Nashville warbler on 12 May 2009 at Sibley Peninsula, Ontario (Site 11); these host records provide the first indication of *I. scapularis* on these 2 bird species. Three undetermined immature *Ixodes* spp. ticks are being held with the anticipation that the nymphal stages for these Neotropical tick species are described in the future.

In addition, during the present study, *Amblyomma* spp. ticks were transported long distances by Neotropical and southern temperate songbirds: *Amblyomma imitator* Kohls is indigenous in Central America and southern Texas; *Amblyomma longirostre* (Koch) is native in northern South America and Central America; and *Amblyomma maculatum* Koch (Gulf Coast tick) occurs naturally in the southern United States, Caribbean region, Central America, and northern South America (Estrada-Peña et al., 2005).

Spirochete detection

During a 2-yr-period (2008–2009), PCR detected *B. burgdorferi* in 52 (29.5%) of 176 ticks tested (Table II). The infection prevalence of *B. burgdorferi* in *I. scapularis* was 36% (nymphs, 31%; larvae, 48%). By province, the biogeographical distribution of *B. burgdorferi*–infected *Ixodes* ticks was as follows: New Brunswick, 1; Quebec, 4; Ontario, 19 (Long Point, 6; Cayuga, 2; Sibley Peninsula, 5; Toronto, 6); Manitoba, 4; Saskatchewan, 1; and British Columbia, 23. In the Maritimes, 1 of 2 *I. scapularis* nymphs collected from a Swainson's thrush on 6 June 2008 in north-central New Brunswick represents the first *B. burgdorferi*–infected tick on a songbird in this elevated, Acadian forest region. In central Canada, an *I. scapularis* nymph was collected from a common yellowthroat on 30 May 2008 on Sibley Peninsula, Ontario (Site 11); this collection constitutes the first report of a *B. burgdorferi*–positive tick on a bird in northwestern Ontario. In the Prairie Provinces, a nymphal *I. scapularis* was removed from a Swainson's thrush after a window strike on 31 May 2009 at

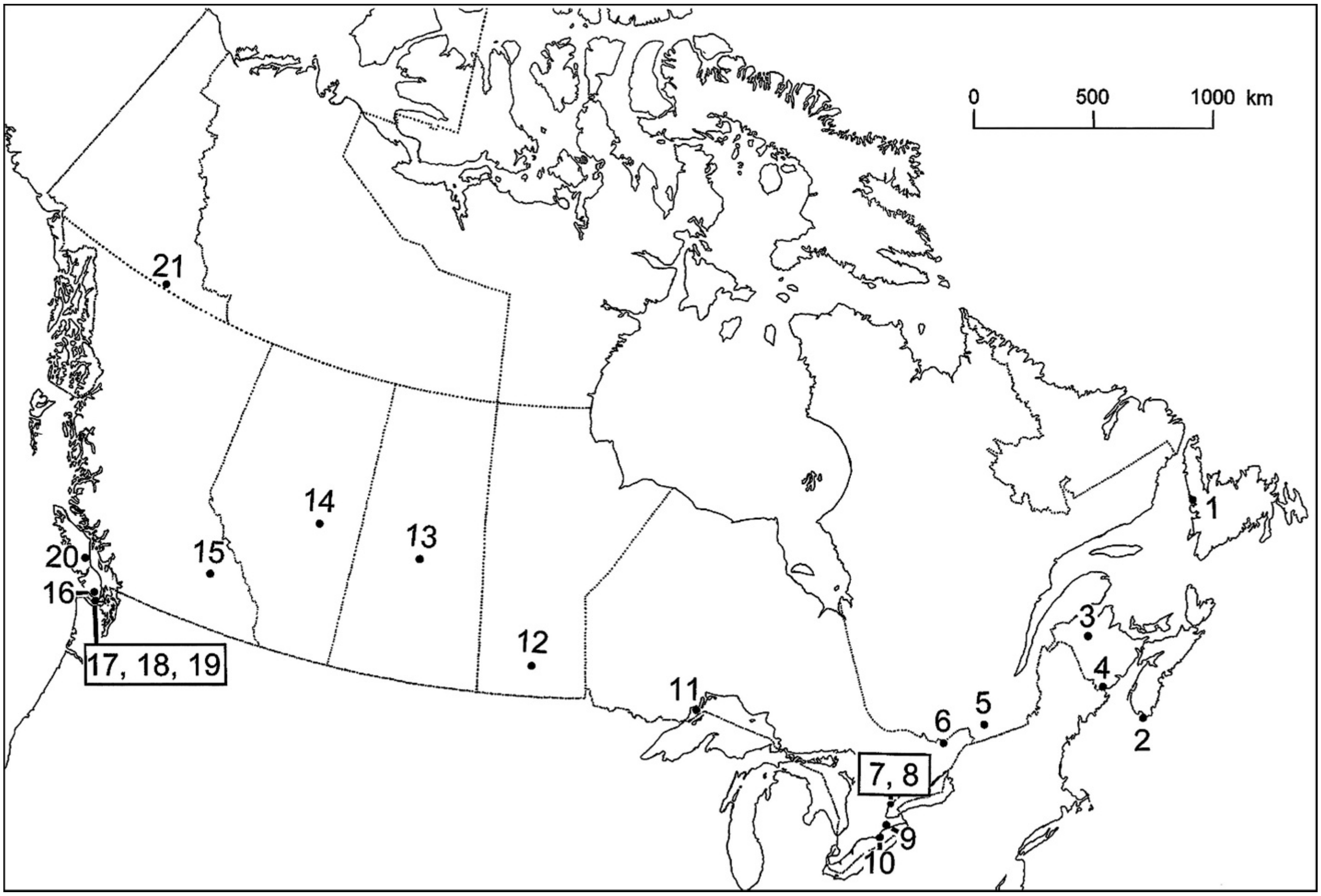

FIGURE 1. Map of Canada showing tick collection sites. (1) MAPS Banding Station, Lobster Cove, Rocky Harbour, Newfoundland and Labrador; 49.64N, 57.98W. (2) Atlantic Bird Observatory, Bon Portage Island, Nova Scotia; 43.46N, 65.74W. (3) Christmas Mountains, New Brunswick; 47.21N, 66.67W. (4) St. Andrews Bird Banding Station, St. Andrews, New Brunswick; 45.68N, 67.06W. (5) McGill Bird Observatory, Ste-Anne-de-Bellevue, Quebec; 45.43N, 73.94W. (6) Innis Point Bird Observatory, Kanata, Ontario; 45.48N; 76.37W. (7) Tommy Thompson Park Bird Research Station,Toronto, Ontario; 43.63N, 79.33W. (8) Fatal Light Awareness Program, Toronto, Ontario; 43.74N, 79.37W. (9) Ruthven Park National Historic Site Banding Station, Haldimand Bird Observatory, Cayuga, Ontario; 42.97N, 79.87W. (10) Long Point Bird Observatory, Long Point (Port Rowan), Ontario; 42.52N, 80.17W. (11) Thunder Cape Bird Observatory, Sibley Peninsula (Thunder Bay), Ontario; 48.42N, 88.78W. (12) Delta Marsh Bird Observatory, Delta Marsh (R.R. #2, Portage la Prairie), Manitoba; 50.18N, 98.20W. (13) Tweedsmuir, Saskatchewan; 53.57N, 105W. (14) Beaverhill Bird Observatory, Tofield, Alberta; 53.37N, 112.52W. (15) Revelstoke, British Columbia; 50.99N, 118.19W. (16) Maltby Lake, Saanich Peninsula, Vancouver Island, B.C.; 48.49N, 123.45W. (17) Willy's Lagoon, Metchosin, Vancouver Island, B.C.; 48.37N, 123.52W. (18) Rocky Point Bird Observatory, Metchosin, Vancouver Is., B.C.; 48.32N, 123.53W. (19) East Sooke, Vancouver Is., B.C.; 48.36N, 123.68W. (20) Mountainaire Avian Rescue Society (Courtenay), Vancouver Is., B.C.; 49.41N, 124.97W. (21) Albert Creek Bird Observatory, Watson Lake, Yukon; 60.07N, 128.92W. Mailing addresses/local urban centers are listed in parentheses.

Tweedsmuir, Saskatchewan (Site 13); this is the first record of a *B. burgdorferi*–infected tick on a bird in this province and, likewise, the first account of a tick on a bird in this province. This fully engorged nymph molted to an adult (female) in 65 days. In addition, we detected *B. burgdorferi* in 2 (40%) of 5 bird-derived *I. dentatus* larvae.

On the west coast, the *B. burgdorferi* infection prevalence for *I. auritulus* was 33% (females, 33%; nymphs, 20%; larvae, 59%). We provide the first record of a *B. burgdorferi*–positive tick (*I. auritulus* female) on a Bewick's wren; it was detached on 9 September 2008 at Rocky Point, B.C. (Site 18). The following year, a fully engorged *I. auritulus* larva was collected from a Bewick's wren on 25 May 2009 at Maltby Lake, B.C. This tick successfully molted to a nymph in 61 days and, during the molt, transstadially passed *B. burgdorferi*.

We provide the first reports of *I. spinipalpis* on songbirds on Vancouver Island, B.C. An *I. spinipalpis* larva was collected from a song sparrow, a short-distance migrant, on 18 April 2009 at Maltby Lake, B.C. (Site 16). In addition, this collection represents the first detection of *B. burgdorferi* in *I. spinipalpis* collected from a bird anywhere. Interestingly, 3 different tick species (*I. auritulus*, *I. pacificus*, and *I. spinipalpis*) co-infested this song sparrow. Likewise, at the same site and same day, a *B. burgdorferi*–positive *I. spinipalpis* nymph was collected from another song sparrow.

DISCUSSION

Our study focuses on bird-tick-*Borrelia* zoonoses and provides many firsts for ixodid ticks on passerines across Canada. Overall, we found the presence of *B. burgdorferi* in 29.5% of *Ixodes* ticks

TABLE I. Ixodid ticks collected from songbirds in Canada during the 3-yr period 2007–2009. Bird nomenclature follows the check-list of the American Ornithologists' Union website: www.aou.org/checklist/index.*php3*. Abbreviations: *bru., brunneus; dentat., dentatus;* spp., undermined species of *Ixodes. Hae. lepo., Haemaphysalis leporispalustris; imit., imitator; long., longirostre; mac., maculatum;* L, larva(e); N, nymph(s); F, female(s).

	Ixodes																	*Hae. lepo.*		*Amblyomma*			Total no. of ticks
	affinis		*auritulus*			*bru.*	*dentat.*		*muris*		*pacificus*		*scapularis*		*spinipalpis*		spp.			*imit.*	*long.*	*mac.*	
Bird species	L	N	L	N	F	F	L	N	L	F	L	N	L	N	L	N	N	L	N	N	L	L	
Swainson's thrush	3	1	1	1	3	—	4	1	—	1	1	—	4	18	—	1	—	68	8	—	—	—	115
Song sparrow	—	—	2	11	2	—	1	1	5	1	1	1	1	—	1	2	—	67	13	—	—	—	109
Common yellowthroat		—	—	—	—	—	—	—	—	2	—	—	2	23	—	—	—	2	—	—	—	—	29
White-throated sparrow	—	—	—	—	—	2	—	1	—	—	—	—	5	5	—	—	—	11	5	—	—	—	29
American robin	—	—	—	3	1	—	—	—	—	—	—	—	—	—	—	—	—	11	6	—	—	—	21
Swamp sparrow	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	14	2	—	—	—	19
Hermit thrush	—	—	—	—	—	1	3	1	—	—	—	—	—	2	—	1	1	4	1	—	—	1	15
Oregon junco*	—	—	9	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15
Fox sparrow	—	—	—	10	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15
Bewick's wren	—	—	3	2	2	—	—	—	—	—	1	—	—	—	2	1	—	—	—	—	—	—	11
Chipping sparrow	—	—	—	—	—	—	—	—	—	1	—	—	—	5	—	—	—	3	2	—	—	—	11
House wren	—	—	—	—	—	—	—	—	—	1	—	—	1	7	—	—	—	—	—	—	—	—	9
Brown-headed cowbird	—	—	—	—	—	—	3	1	—	—	—	—	—	3	—	—	—	1	—	—	—	—	8
Spotted towhee	—	—	—	4	—	—	—	—	—	—	—	—	—	—	1	3	—	—	—	—	—	—	8
Northern waterthrush	—	—	—	—	—	—	—	—	—	—	—	—	2	1	—	—	—	2	1	—	—	—	6
Dark-eyed junco	—	—	—	—	—	—	—	—	—	—	—	1	3	1	—	—	1	—	—	—	—	—	6
Lincoln's sparrow	—	—	—	—	—	—	—	—	—	1	—	—	—	—	2	—	1	—	1	—	—	—	5
Veery	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	1	1	—	—	—	4
Northern cardinal	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	3	—	—	—	—	4
Eastern white-crowned sparrow	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	3
Mourning warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	3
Ovenbird	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	3
Gray catbird	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	1	—	—	—	—	3
Wilson's warbler	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
Indigo bunting	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	3

(Table I continued)

TABLE I. Continued.

	Ixodes																	*Hae.*		*Amblyomma*			Total no. of ticks
	affinis		*auritulus*			*bru.*	*dentat.*		*muris*		*pacificus*		*scapularis*		*spinipalpis*		spp.	*lepo.*		*imit.*	*long.*	*mac.*	
Bird species	L	N	L	N	F	F	L	N	L	F	L	N	L	N	L	N	N	L	N	N	L	L	
Gray-cheeked thrush	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—	1	3
Red-winged blackbird	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	2
Varied thrush	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	2
Canada warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	2
Pine siskin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	2
Nashville warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	2
Nashville warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	2
Black-and-white warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Blackpoll warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
American redstart	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
Winter wren	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
Purple finch	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
Brown thrasher	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Blue jay	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Golden-crowned sparrow	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Orange-crowned warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
Yellow warbler	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Pacific wren†	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Total (42 bird species)	3	1	19	35	16	6	11	6	5	7	3	2	22	86	8	8	3	193	41	1	3	2	481

* Oregon junco, which is a subspecies of the dark-eyed junco, is common in western Canada, especially in British Columbia.

† This newly named species was formerly considered the winter wren in western North America.

TABLE II. Detection of *Borrelia burgdorferi* in tested *Ixodes* ticks collected from songbirds across Canada during the 2-yr period 2008–2009. Bird nomenclature follows the check-list of the American Ornithologists' Union website: www.aou.org/checklist/index.*php3*. Abberviations: L, larva(e); N, nymph(s); F, female(s): *bru., brunneus.*

	No. positive/no. tested														
	I. auritulus			*I. bru.*	*I. dentatus*		*I. muris*		*I. pacificus*		*I. scapularis*		*I. spinipalpis*		Total no. positive/ no. tested
Bird species	L	N	F	F	L	N	L	F	L	N	L	N	L	N	
Swainson's thrush	2/3	1/1	1/2	—	2/4	0/1	—	0/1	0/1	—	1/3	5/11	—	0/2	12/29
Song sparrow	2/2	3/10	0/2	—	0/1	0/1	0/3	0/1	—	0/2	—	0/1	1/1	1/1	7/25
Bewick's wren	3/3	1/2	1/2	—	—	—	—	—	0/1	—	—	—	1/1	0/1	6/10
Common yellowthroat	—	—	—	—	—	—	—	0/2	—	—	2/7	4/14	—	—	6/23
Oregon junco	3/7	0/4	2/2	—	—	—	—	—	—	—	—	—	—	—	5/13
White-throated sparrow	—	—	—	0/1	—	—	—	—	—	—	3/5	0/4	—	—	3/10
Swamp sparrow	—	—	—	—	—	—	—	—	—	—	2/2	—	—	—	2/2
Nashville warbler	—	—	—	—	—	—	—	—	—	—	—	2/2	—	—	2/2
Fox sparrow	—	2/10	0/3	—	—	—	—	—	—	—	—	—	—	—	2/13
Red-winged blackbird	—	—	—	—	—	—	—	0/1	—	—	1/1	—	—	—	1/2
Ovenbird	—	—	—	—	—	—	—	—	—	—	—	1/2	—	—	1/2
Hermit thrush	—	—	—	0/1	—	—	—	—	—	—	—	1/1	—	—	1/2
Indigo bunting	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	1/3
House wren	—	—	—	—	—	—	—	0/1	—	—	—	1/2	—	—	1/3
Chipping sparrow	—	—	—	—	—	—	—	—	—	—	—	1/4	—	—	1/4
Dark-eyed junco	—	—	—	—	—	—	—	—	—	—	1/4	0/1	—	—	1/5
Yellow warbler	—	—	—	—	—	—	—	—	—	—	—	0/1	—	—	0/1
Gray-cheeked thrush	—	—	—	—	—	—	—	—	—	—	—	0/1	—	—	0/1
Pacific wren	0/1	—	—	—	—	—	—	—	—	—	—	—	—	—	0/1
Orange-crowned warbler	—	—	—	—	—	—	—	—	—	—	—	—	0/1	—	0/1
Wilson's warbler	0/1	—	—	—	—	—	—	—	—	—	—	—	—	—	0/1
Northern waterthrush	—	—	—	—	—	—	—	—	—	—	—	0/1	—	—	0/1
Mourning warbler	—	—	—	—	—	—	—	—	—	—	—	0/2	—	—	0/2
Gray catbird	—	—	—	—	—	—	—	—	—	—	—	02	—	—	0/2
Lincoln's sparrow	—	—	—	—	—	—	—	0/1	—	—	—	—	0/2	—	0/3
American robin	—	0/3	0/1	—	—	—	—	—	—	—	—	—	—	—	0/4
Spotted towhee	—	0/4	—	—	—	—	—	—	—	—	—	—	—	0/4	0/8
	10/17	7/34	4/12	0/2	2/5	0/2	0/3	0/7	0/2	0/2	10/22	16/52	2/5	1/8	52/176*

* Total (176) includes 3 *Ixodes affinis* larvae on a Swainson's thrush, which all tested negative for *B. burgdorferi.*

tested. Dispersal of *I. scapularis* was evident east of the Rockies, and *B. burgdorferi* prevalences of 48% and 31% were detected in larvae and nymphs, respectively. Our elevated *B. burgdorferi* infection prevalences, especially by larvae, suggest that certain passerine hosts may maintain spirochetemia and are competent reservoirs. In addition, collections of *B. burgdorferi*–infected larvae during avian nesting and juvenile development indicate established populations of Lyme vector ticks in certain locales, or bird-to-tick transmission of Lyme disease spirochetes. Ground-foraging passerines, i.e., juncos, sparrows, thrushes, warblers, and wrens, are the predominant hosts of bird-associated ticks, and some spirochetemic songbirds can potentially initiate new Lyme

FIGURE 2. Hermit thrush parasitized by an engorged nymph of the rabbit tick, *Haemaphysalis leporispalustris*. Photo credit: Paloma Plant.

disease–endemic foci. Combining the present study and a previous pan-Canadian study (Scott et al., 2010), we have detected *B. burgdorferi* in 6 different bird-feeding *Ixodes* ticks. The collection of *I. affinis* in Manitoba, *I. auritulus* in the Yukon, and *Amblyomma* spp. ticks in Ontario illustrates the fact that migratory songbirds carry ticks thousands of km. Based on our high prevalence data for *B. burgdorferi*, outdoors people need to examine themselves carefully after frequenting grassy and woody habitats. Clinically, doctors need to be alert to the possibility that the ticks, which are discovered, might be infected, and patients treated accordingly (Oksi et al., 1999; Miklossy, 2008). Also, doctors should be aware that infection could happen almost anywhere in Canada.

Extended dispersal of *I. auritulus* ticks in the Yukon

Every spring, songbirds migrate to Canada's northland to breed and nest in the boreal forest. At Watson Lake, Yukon, 2 engorged *I. auritulus* larvae were collected on 11 May 2008 from a Wilson's warbler (subspecies *pileolata*, which breeds in the Pacific Northwest, including the Yukon). Since there are no known inland populations of *I. auritulus*, we believe that these *I. auritulus* larvae may have originated from the eastern seaboard of Mexico; this tick species normally inhabits the east coast of Central America and the Pacific coast (Durden and Keirans, 1996; Robbins et al., 2001). En route to the Yukon, the Wilson's warbler, which mainly winters in Mexico and Central America, most likely followed the eastern face of the Rocky Mountains as its migratory corridor (Dunn and Garrett, 1997). Our findings correspond with tick recoveries in 1942 when larval and nymphal *I. auritulus* were collected during spring migration from a white-throated swift, *Aeronautes saxatilis* (Woodhouse) (Apodiformes), in Colorado (Cooley and Kohls, 1945); these *I. auritulus* collections were also located along the central flyway. Our extralimital tick findings are the most northern account (north of 60th latitude) of *I. auritulus* in Canada and also the first report of this tick species in the Yukon.

Flight path of Swainson's thrush carrying 3 tick species

We provide the first-ever documentation of 3 tick species cofeeding on a songbird. In total, 4 *I. affinis* (1 nymph, 3 larvae), 4 *I. dentatus* larvae, and 1 *I. scapularis* nymph were collected from a Swainson's thrush, a Neotropical migrant, on 27 May 2008 at Delta Marsh, Manitoba (Table I, Fig. 2). Based on the indigenous areas of these 3 different tick species, we were able to hypothesize the flight path. Because *I. affinis* has the most southern natural geographical range (coastal and sub-coastal Florida, Georgia, South Carolina, and North Carolina) (Harrison et al., 2010), the immatures of this tick species would attach first. Later, during the northward flight, the Swainson's thrush was most likely parasitized by *I. dentatus* and *I. scapularis* in the north-central United States. This avian host is much more likely to have been infested in the upper Midwest where endemicity of *B. burgdorferi* is elevated, and the flight path is directly in line with Delta Marsh. This flight path is consistent with endpoints of previous recovery data for spring migration patterns of Swainson's thrush (Brewer et al., 2000). Both *I. dentatus* and *I. scapularis* are indigenous in the upper Midwest and act as vectors in bird-rabbit enzootic transmission cycles of *B. burgdorferi* (Durden and Keirans, 1996; Oliver et al., 1998). Despite *I. affinis* being a competent vector for *B. burgdorferi*, all of the attached immatures were negative. In contrast, 1 *I. scapularis* nymph and 2 *I. dentatus* larvae were infected with *B. burgdorferi*. Most likely, cross-transmission of spirochetes occurred during cofeeding between the *I. scapularis* nymph and 2 of 4 *I. dentatus* larvae via endogenous transmission (within the host bird). For example, larvae of *Ixodes ricinus* L. became infected with *B. burgdorferi* while cofeeding in proximity to infected nymphs on hosts that were initially spirochete-free (Gern and Rais, 1996). As well, co-transmission (within a single tick species) of Lyme disease spirochetes could have occurred between each of the attached *I. dentatus* larvae by hematogenous transmission via the host bird. Because transovarial transmission of *B. burgdorferi* is extremely rare and also these larvae had previously not attached to a host, these 2 *I. dentatus* larvae almost certainly acquired infection from the Swainson's thrush (Oliver et al., 1998). Of special note, this is the first record of a *B. burgdorferi*–infected *I. dentatus* in Manitoba collected from a bird. Although an uncommon phenomenon, *I. dentatus* has been known to attach to humans (Soller, 1955; Harrison et al., 1997). Not only is this the first-ever report of 3 tick species simultaneously feeding on a bird, it projects a migratory flight path for Swainson's thrush and clearly exemplifies cross-border movement of ticks into Canada.

Transstadial transmission of *B. burgdorferi* in *I. auritulus*

We provide the first record of transstadial transmission of *B. burgdorferi* in *I. auritulus*. An engorged *I. auritulus* larva, which detached from a Bewick's wren on 25 May 2009 at Maltby Lake, B.C., molted to a nymph in 61 days; it later tested positive for *B. burgdorferi*. Even though *I. auritulus* does not bite humans, it may act as an important enzootic vector of the spirochetes along coastal B.C. During our study, we detected *B. burgdorferi* in all 3 motile developmental life stages (larva, nymph, adult) of *I. auritulus* (Table II). Not only is *B. burgdorferi* transmitted from larvae to nymphs, we hypothesize that transstadial transmission also occurs in the nymph-adult molt of *I. auritulus*.

Four tick enzootic cycle of *B. burgdorferi* in British Columbia

Based on our findings, we hypothesize that a 4-tick enzootic cycle of Lyme disease spirochetes is present on southern

Vancouver Island, B.C. Previously tick-host-pathogen studies have reported *Ixodes angustus* Neumann (a small mammal tick) and *I. pacificus* on deer mice, *Peromyscus maniculatus* (Wagner), which is a natural reservoir of *B. burgdorferi* (Banerjee et al., 1994). Additionally, Banerjee et al. (1994) cultured *B. burgdorferi* from *I. angustus* and *I. pacificus*, and organs of host *P. maniculatus* collected in the Salish Sea area, including southern Vancouver Island. In the present study, we detected *B. burgdorferi* in bird-derived *I. auritulus* and *I. spinipalpis* and, likewise, in *I. pacificus* previously collected from songbirds in southwestern B.C. (Scott et al., 2010). Notably, 3 sympatric tick species (*I. auritulus*, *I. pacificus*, and *I. spinipalpis*) were collected at Maltby Lake, B.C., on 18 April 2009. Because immatures of *I. auritulus* and *I. spinipalpis* were infected with *B. burgdorferi*, these tick infections demonstrate the possibility of spirochete transmission via a vertebrate host between these tick species. *Ixodes auritulus* was collected from April to October, and we believe this tick species may play an important maintenance role in the enzootic transmission cycle of *B. burgdorferi* via songbird parasitism. In this locality both *I. pacificus* and *I. spinipalpis* have the potential to act as a bridge vector to humans. Although not commonly reported, *I. spinipalpis* has been collected from humans (Gregson, 1956; Eisen et al., 2006). Each of these 3 bird-feeding tick species is capable of transmitting *B. burgdorferi* to non-bird hosts, and, thus, Lyme disease spirochetes can be maintained within 2 or more contiguous, enzootic cycles forming a complex web-like network. The propensity of *B. burgdorferi* to survive in all 3 post-embryonic life stages of *I. auritulus* highlights the need for additional studies, especially to determine (1) transovarial transmission of *B. burgdorferi* and (2) the possibility that this tick parasitizes small mammals in this coastal bioregion.

Songbirds as initiators of Lyme disease endemic areas

Songbirds can act as vanguards for new populations of ticks. During biannual migration, songbirds disperse millions of Lyme disease vector ticks across Canada, some of which harbor bacterial microbes (Morshed et al., 2005; Ogden et al., 2008; Scott et al., 2010). During migratory flight, songbirds make landfall to rest, reenergize, and replenish fat reserves and, as a consequence, can acquire Lyme disease vector ticks, especially when these migrants transect endemic areas. Based on *B. burgdorferi* prevalences of 15.4% and 0% for *I. scapularis* nymphs and larvae during spring migrations, respectively, Ogden et al. (2008) speculate that *I. scapularis*–infested songbirds lack the intrinsic tendency to start an endemic area for *B. burgdorferi*. Contrary to their findings, we obtained 36% prevalence for *B. burgdorferi* in *I. scapularis* (nymphs, 31%; larvae, 48%) (Table II), and we contend that *I. scapularis*–infested songbirds can initiate new Lyme-endemic areas. Moreover, Brinkerhoff et al. (2011) report a *B. burgdorferi* infection prevalence of 37.9% in the Midwest for bird-derived *I. scapularis* larvae, which is comparable with our findings.

Songbirds have the potential to be infested by a large numbers of ticks and, after tick engorgement, release them at a single location. For example, Anderson and Magnarelli (1984) removed 21 *I. scapularis* from a gray catbird and a swamp sparrow and, likewise, 19 nymphs from an American robin at the time of capture. These ticks can be transported by seasonal migrants to northern localities during spring migration and released in 1 location, or this heavy infestation of ticks could be dropped locally where they were acquired. In our study, 52 immature *H. leporispalustris* ticks were collected from a Swainson's thrush and, thus, exhibit high infestation. Birds sometimes eat ticks (Stafford and Kitron, 2002) and, at the resting spot of a gravid female carcass, ground-foraging songbirds may become heavily infested by newly developed, questing larvae. Subsequently, these engorged larval ticks can be released in a single locality. Based on the elevated *B. burgdorferi* infection prevalences of immature *I. scapularis* (36%) and *I. auritulus* (33%), our data draw us to the conclusion that songbirds can facilitate new foci of spirochete-bearing ticks.

Endemicity correlates with songbird flyways and connects with intercontinental flight

Many of our collection sites were located primarily on migratory pathways for passerine birds, and assisted in pinpointing the origins of spirochetal infections. Villeneuve et al. (2011) conducted a nationwide canine study of heartworm and 3 tick-transmitted pathogens and found a seroprevalence of 0.72% in dogs tested for Lyme disease. Although it was not noted by these authors, areas of highest prevalence of Lyme disease corresponded with 3 of 4 major songbird flyways. With the exception of the central flyway, 3 flyways correspond to high prevalence of Lyme disease, i.e., eastern Canada (Maritimes, southwestern Quebec, southern Ontario) are extensions of the Atlantic flyway; central Canada (northwestern Ontario and southern Manitoba) are extensions of Mississippi flyway; and southwestern B.C. coincides with the Pacific flyway. The only known Lyme-endemic area along the central flyway is a population of *I. spinipalpis* in northern Colorado (Schneider et al., 2000).

Migratory songbirds have a great potential to transport ticks long distances, to and from, the Neotropics. Of particular note, we report the first bird parasitism by *I. scapularis* on Blackpoll warbler, a Neotropical songbird. During fall migration, these birds embark on a marathon flight from the eastern seaboard of Atlantic Canada and the northeastern United States, and fly south nonstop over water for up to 88 hr to wintering grounds in Puerto Rico and northern South America, a distance of 3,000 to 3,500 km (Hunt and Elaison, 1999); these passerines may be parasitized by *B. burgdorferi*–infected ticks during the southward fall migration. Alternatively, Neotropical migrants wintering in Brazil and surrounding countries are able to transport spirochete-infected ticks to North America during northward spring migration. A Brazilian tick-borne disease caused by *B. burgdorferi* has clinical symptoms similar to those of Lyme disease in northern temperate zones (Gouveia et al., 2010), and this borrelial genotype could be transported to Canada by bird-transported ticks during intercontinental flight.

Songbirds act as competent reservoirs of *B. burgdorferi*

When Lyme disease vector ticks take a blood meal from *B. burgdorferi*–infected songbirds, they can subsequently transmit spirochetes to suitable vertebrate hosts (Anderson et al., 1990; Richter et al., 2000). Some ground-foraging songbirds harbor borreliae, and these avian hosts are often overlooked as important reservoirs of *B. burgdorferi*. Based on a comprehensive study using spirochete-free, xenodiagnostic larvae, Richter et al. (2000) found that the American robin, which is the most common native songbird in Canada, is a competent reservoir for *B. burgdorferi.* During pioneer borrelial studies in North America, Anderson et al. (1986) isolated *B. burgdorferi* from the liver of a veery and, likewise, cultured Lyme disease spirochetes from the blood of an American robin and common yellowthroat, and also from attached *I. scapularis* larvae (Anderson and Magnarelli, 1984). Since transovarial transmission of *B. burgdorferi* is extremely rare in wild-caught *I. scapularis* larvae (Magnarelli et al., 1987; Patrican, 1997), larvae almost certainly acquired borreliae directly from certain passerines. Overall, 9 different passerine species/subspecies, i.e., Bewick's wren, common yellowthroat, dark-eyed junco, Oregon junco, red-winged blackbird, song sparrow, Swainson's thrush, swamp sparrow, and white-throated sparrow, in our study were parasitized by *B. burgdorferi*–infected *Ixodes* larvae, which suggests these avian hosts are competent reservoirs. Additionally, during a prior bird-tick-borrelial study across Canada, Scott et al. (2010) detected *B. burgdorferi*–infected larvae on Swainson's thrushes, common yellowthroats, and song sparrows. Implicitly, the elevated infection prevalence of Lyme disease spirochetes in bird-derived larvae shows that several passerine species maintain spirochetemia, and act as competent-reservoir hosts (Table II). Moreover, collections of *B. burgdorferi*–infected *I. scapularis* larvae in mid-August indicate that these passerine hosts may be spirochemic for 3 mo or longer.

Anderson et al. (1990) reported the first isolation of *B. burgdorferi* from a bird-feeding larval *I. dentatus*. Other studies also demonstrate that this tick species is involved in bird-rabbit cycles of *B. burgdorferi* (Anderson et al., 1989; Kollars and Oliver, 2003). Recently, Hamer et al. (2011) obtained diverse *B. burgdorferi* strains in immature *I. dentatus* collected from songbirds in Michigan. Analogous to these studies, we detected Lyme disease spirochetes in 2 *I. dentatus* larvae collected from a Swainson's thrush. Normally, *I. dentatus* do not bite people, but parasitism of humans has been reported (Sollers, 1955; Anderson et al., 1996).

Impact of bird-associated ticks on humans

Lyme disease can produce a myriad of symptoms, including endocrine, cardiac, musculosketal, cutaneous, ocular, cognitive, neuropsychiatric, urogenital, and gastrointestinal symptoms (Maloney, 2009; Savely, 2010). Eventually, it can develop into a chronic, debilitating disease for mo, or years, with recurrent bouts of arthritis and an array of neurological manifestations (Liegner et al., 1993; Preac-Mursic et al., 1993; Oksi et al., 1999; Livengood and Gilmore, 2006; Bankhead and Chaconas, 2007; Miklossy, 2008; Cameron, 2010). If left untreated, Lyme disease spirochetes can invade virtually all body tissues and allow Lyme disease to advance to the latent stage, causing persistent, irreversible symptoms that have a profound impact on patients. In some cases, patients have fatal outcomes (Preac-Mursic et al., 1993; Tavora et al., 2008). Congenital Lyme disease has also been reported (MacDonald, 1989; Gardner, 2001).

With more than 50 *B. burgdorferi* genotypes across North America and Eurasia (Crowder et al., 2010), serological diagnostic tests must include local strains to optimize test results. Unfortunately, Canadian testing laboratories have not yet considered geographic and genome sequence variation in *B. burgdorferi* strains. Hopefully, by considering the wide variation of strain variants dispersed by songbirds, diagnostic testing will become more helpful in accurately assessing Lyme disease patients (Sperling and Sperling, 2009; Evans et al., 2010; Jiang et al., 2010). Often clinicians are not aware of the genetic heterogeneity of Lyme disease borreliae and rely on laboratory results that are based solely on the type strain B31. As evident in our bird-tick-*Borrelia* study, multiple divergent strains of *B. burgdorferi* can be transported long distances, and missed during patient serological screening. Optimistically, the present study will help scientists develop more accurate diagnostic methods and appropriate therapies in Canada.

In summary, our study demonstrated that *Borrelia*-infected ticks on songbirds are more widespread and common than was previously documented for Canada. Spirochete-infected larvae on 9 different bird species suggest that certain passerines are competent reservoirs. The presence of 4 different *Ixodes* species ticks, infected with *B. burgdorferi* on southern Vancouver Island, B.C., suggests a 4-tick enzootic transmission cycle that encompasses a community of interacting vector ticks and vertebrate hosts, including humans. The first discovery of *I. affinis* in Canada and, likewise, *I. auritulus* in the Yukon underscore the fact that songbirds disperse vector ticks during long-distance migratory flight. Multiple ticks on a single bird show that songbirds that are parasitized with *B. burgdorferi*–infected ticks have the potential to initiate new tick populations endemic for Lyme disease. The Canadian medical profession has had a long-standing belief that people must visit an endemic area to be bitten by an infected tick; however, we show that songbirds play a pivotal role in the wide dispersal of Lyme disease–carrying ticks across Canada. Because songbirds disperse vector ticks over a broad geographic area, travel to an endemic area should be excluded from Lyme disease surveillance criteria. Millions of *B. burgdorferi*–infected ticks are widely dispersed across Canadian geographic regions and clearly pose a public health risk.

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