Detection of Lyme disease spirochete, *Borrelia burgdorferi* sensu lato, including three novel genotypes in ticks (Acari: Ixodidae) collected from songbirds (Passeriformes) across Canada

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ABSTRACT: Lyme disease is reported across Canada, but pinpointing the source of infection has been problematic. In this three-year, bird-tick-pathogen study (2004-2006), 366 ticks representing 12 species were collected from 151 songbirds (31 passerine species/subspecies) at 16 locations Canada-wide. Of the 167 ticks/pools tested, 19 (11.4%) were infected with *Borrelia burgdorferi* sensu lato (s.l.). Sequencing of the *rrf-rrl* intergenic spacer gene revealed four *Borrelia* genotypes: *B. burgdorferi* sensu stricto (s.s.) and three novel genotypes (BC genotype 1, BC genotype 2, BC genotype 3). All four genotypes were detected in spirochete-infected *Ixodes auritulus* (females, nymphs, larvae) suggesting this tick species is a vector for *B. burgdorferi* s.l. We provide first-time records for: ticks in the Yukon (north of 60° latitude), northernmost collection of *Amblyomma americanum* in North America, and *Amblyomma imitator* in Canada. First reports of bird-derived ticks infected with *B. burgdorferi* s.l. include: live culture of spirochetes from *Ixodes pacificus* (nymph) plus detection in *I. auritulus* nymphs, *Ixodes scapularis* in New Brunswick, and an *I. scapularis* larva in Canada. We provide the first account of *B. burgdorferi* s. l. in an *Ixodes muris* tick collected from a songbird anywhere. Congruent with previous data for the American Robin, we suggest that the Common Yellowthroat, Golden-crowned Sparrow, Song Sparrow, and Swainson's Thrush are reservoir-competent hosts. Song Sparrows, the predominant hosts, were parasitized by *I. auritulus* harboring all four *Borrelia* genotypes. Our results show that songbirds import *B. burgdorferi* s.l.-infected ticks into Canada. Bird-feeding *I. scapularis* subadults were infected with Lyme spirochetes during both spring and fall migration in eastern Canada. Because songbirds disperse millions of infected ticks across Canada, people and domestic animals contract Lyme disease outside of the known and expected range. ***Journal of Vector Ecology* 35 (1): 124-139. 2010.**

Keyword Index: Birds, *Borrelia burgdorferi*, Lyme disease, ticks, *Ixodes*, Canada.

INTRODUCTION

The source of sporadic Lyme disease (Lyme borreliosis) across Canada has been a mystery. Several tenets abound about the origin of this bacterial illness, but science is often lacking. The Lyme disease spirochete, *Borrelia burgdorferi* sensu lato (s.l.), has been found in established populations of vector ticks along the southern fringe of Canada; however, people contract this zoonosis hundreds of kilometers from these tick foci. In Ontario, the province reporting the highest incidence of human Lyme disease, 85% of locally acquired cases (2002-2006) had no exposure in an endemic area (MHLTC 2007). By and large, songbirds (Passeriformes) have been overlooked by the Canadian public in the epidemiology of Lyme disease.

Historically, bird-parasite studies 50 years ago in Sudan and Egypt described ticks on spring- and autumn-migrating landbirds flying between Eurasia and Africa (Hoogstraal and Kaiser 1961). Ticks are transported long distances within and between continents by birds (Olsén et al. 1995, Durden et al. 2001, Reed et al. 2003, Scott and Durden 2009). Ectoparasitic ticks infected with *Borrelia burgdorferi* s.l. have been reported on songbirds across the northern hemisphere (Anderson et al. 1990, Alekseev et al. 2001, Livanova et al. 2003, Comstedt et al. 2006, Wright et al. 2006). Because songbirds are highly mobile, they have exceptional capacity to serve as dispersal hosts and, ultimately, introduce infected ticks.

Borrelia burgdorferi s.l., initially considered as a single species, consists of at least 15 recognized genospecies: *B. afzelii*, *B. andersonii*, *B. bissettii*, *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, *B. japonica*, *B. lusitaniae*, *B. sinica*, *B. spielmanii*, *B. tanukii*, *B. turdae*, *B. valaisiana* and, lately, *B. californiensis* (Postic et al. 2007), *B. carolinensis* (Rudenko et al. 2009a), and *B. americana* (Rudenko et al. 2009b). Collectively, this divergent group of *B. burgdorferi* s.l. has been isolated from at least 17 species of ixodid ticks in two genera (15 *Ixodes* spp., two *Haemaphysalis* spp.) primarily

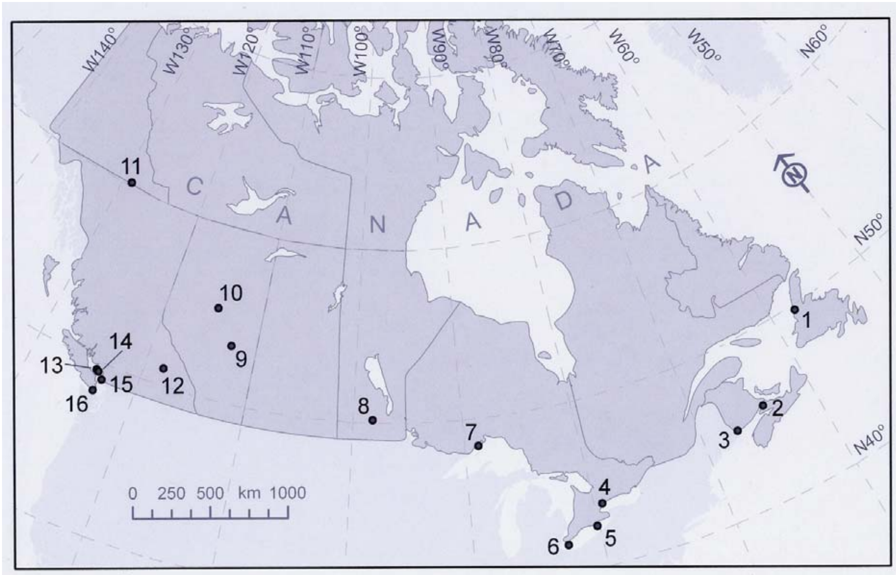

Figure 1. Sites in Canada where ticks were collected from songbirds. 1. Gros Morne National Park Migration Station, Lobster Cove Head, Newfoundland and Labrador; Latitude: N49.69, Longitude: W57.74. 2. Amherst Point, Nova Scotia; N45.75, W64.27. 3. Huntsman Marine Science Centre, St. Andrews, New Brunswick; N45.08, W67.06. 4. Fatal Light Awareness Program, Toronto, Ontario; N43.74, W79.37. 5. Long Point Bird Observatory, Long Point (Port Rowan), Ontario; N42.52, W80.17. 6. Pelee Island Bird Observatory, Pelee Island, Ontario; N41.78, W82.66. 7. Thunder Cape Bird Observatory, Sibley Peninsula (Pass Lake), Ontario; N48.42, W88.78. 8. Delta Marsh Bird Observatory, Delta (Portage la Prairie), Manitoba; N50.18, W98.20. 9. Beaverhill Bird Observatory, Tofield, Alberta; N53.37, W112.52. 10. Lesser Slave Lake Bird Observatory, Slave Lake, Alberta; N55.28, W114.77. 11. Albert Creek Bird Observatory, Watson Lake, Yukon; N60.07, W128.92. 12. Revelstoke, British Columbia; N50.98, W118.20. 13. Trout Lake, B.C.; N49.52, W123.92. 14. Wilson Creek, B.C.; N49.45, W123.72. 15. Alsaken National Wildlife Area, Westham Island, B.C.; N49.08, W123.15. 16. Rocky Point Bird Observatory, Metchosin, Vancouver Island, B.C.; N48.32, W123.53. Mailing addresses are listed in parentheses.

from temperate zones of the northern hemisphere.

People become morbidly afflicted hosts within the ecological system of Lyme disease. As this spirochetal infection progresses in the body, patients typically experience cutaneous, musculoskeletal, cardiac, ophthalmological, lymphatic, neurocognitive, and neuropsychological symptoms. Left undiagnosed and untreated, different pleomorphic forms (i.e., spirochetes, blebs, granules, L-forms, cysts), tangles, and microfilms (spirochete colonies) develop, which can linger and inflict a plethora of persistent, debilitating, clinical manifestations (Gardner 2001). The most prevalent genospecies, which have been isolated from patients, are *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii*. All of these pathogens have been cultured from ticks collected from songbirds. Additional genospecies have been detected in and/or isolated from human tissue and are now considered pathogenic, including *B. bissettii* (Bissett and Hill 1987, Picken et al. 1996, Maraspin et al. 2002), *B. lusitaniae* (da Franca et al. 2005), *B. spielmanii* (Richter et al. 2006), and *B. valaisiana* (Diza et al. 2004).

In North America, seven genospecies of *B. burgdorferi* s.l. are associated with a wide range of vectors and hosts: *B. americana* (Rudenko et al. 2009b), *B. andersonii* (Anderson et al. 1989, Oliver et al. 1998, Lin et al. 2004), *B. bissettii* (Anderson et al. 1988, Oliver et al. 1996, Lin et al. 2004, Ullmann et al. 2005), *B. californiensis* (Postic et al. 2007), *B. carolinensis* (Rudenko et al. 2009a), and *B. garinii* (Smith et al. 2006). Continentally, *B. burgdorferi* s.s. is the most commonly occurring genospecies.

Within the Pacific Northwest, at least four tick species act as enzootic vectors of *B. burgdorferi* s.l.: *Ixodes angustus* Neumann (Damrow et al. 1989, Banerjee et al. 1994a, Eisen et al. 2006); *Ixodes spinipalpis* Hadwen and Nuttall (Maupin et al. 1994, Eisen et al. 2003, 2006); *Ixodes auritulus* Neumann (Morshed et al. 2005); and the western blacklegged tick, *Ixodes pacificus* Cooley and Kohls (Burgdorfer et al. 1985, Clover and Lane 1995, Eisen et al. 2003). Initially, Gregson (1956) identified ticks on wild birds in Canada; however, *I. pacificus* was not reported on songbirds. *Ixodes pacificus*, a lizard-, rodent-, and bird-associated tick, is the principal vector of *B. burgdorferi* s.l. in far-western North America (Eisen et al. 2006). Castro and Wright (2007) listed 42 passerine species as avian hosts for *I. pacificus* subadults in California, and Morshed et al. (2005) reported *I. pacificus* on songbirds in the two westernmost Canadian provinces, British Columbia (B.C.) and Alberta. The blacklegged tick, *Ixodes scapularis* Say, is the primary vector of *B. burgdorferi* s.l. east of the Rocky Mountains. Geographically, *I. scapularis* has been collected from songbirds from as far west and as far north as northcentral Alberta (Scott et al. 2001, Morshed et al. 2005).

The present trans-Canadian study was undertaken to (1) determine the presence of *B. burgdorferi* s.l. in ticks infesting songbirds at several locales across Canada, especially at three B.C. coastal sites, (2) compare the genospecies and/or genomic groups of spirochetes in bird-associated ticks, (3) assess the occurrence of tick fauna on avian hosts north of the 60th latitude, and (4) clarify the northern range of ticks carried by spring migrants in the Canadian North.

MATERIALS AND METHODS

Tick collections from songbirds

Ticks were collected by bird banders and wildlife rehabilitators from naturally infested songbirds at 16 locations across Canada (Figure 1) spanning the period April 2004 to October 2006. Collections were conducted primarily in the spring; however, ticks were retrieved throughout the bird banding season (April-October), particularly at three coastal sites in B.C. Bird captures were made by bird banders using Japanese mist nets. Injured or sick birds submitted by the public were examined by wildlife rehabilitators. Birds were casually scanned for ticks while collecting physical measurements and data. Because of the elusive nature of minute, immature ticks (e.g., unengorged *I. scapularis* larva, 0.8 mm), some ticks may have been missed. Readily visible ticks were removed using fine-pointed tweezers, retained in polyethylene vials capped with tulle netting, and placed in a self-sealing plastic bag with moist paper towel. These ticks were identified using a binocular dissecting microscope (×8 to ×40 lens objective) to determine species, developmental life stage, and degree of engorgement. Some of the ticks were sent to Georgia Southern University for identification. A larval key (Clifford et al. 1961) and, likewise, nymphal *Ixodes* (Durden and Keirans 1996) and *Amblyomma* (Keirans and Durden 1998) keys were employed. Larval *I. auritulus* and *A. longirostre* were identified using Webb et al. (1990) and (Barros-Battesti et al. 2002), respectively. In the case of the *A. sabanerae* nymph, voucher specimens were used for morphological comparison and confirmed positive using DNA sequence analysis. A tick occurrence consisted of one tick species on an individual bird. Multiples of each developmental life stage were normally pooled for culturing and testing, and singleton ticks were processed individually. English and scientific nomenclature of bird species is based on the checklist website (http://www.aou.org/checklist/index.php3) of the American Ornithologists' Union. ArcGIS 9.1 (Environmental Systems Research Institute, Redlands, CA) was used to pinpoint sampling areas on the map (Figure 1).

Spirochete detection in ticks

At the British Columbia Centre for Disease Control, live ticks were surface sterilized using 10% hydrogen peroxide followed by 70% ethanol, and rinsed with sterile water. Midgut contents were cultured for mobile borreliae in BSK-H medium. Cultures were incubated at 34° C, and checked weekly for live spirochetes by dark-field microscopy (x400) for 30 days. Some ticks were not tested because they are not typical *Borrelia burgdorferi* s.l. vectors or were of novel significance, and they were retained as voucher specimens at the Lyme Disease Association of Ontario or the United States National Tick Collection (USNTC) at Georgia Southern University, Statesboro, GA.

DNA extraction and PCR amplification

Total DNA was extracted from tick cultures using Qiagen tissue kits (QIAGEN, Mississauga, ON) according to manufacturer's instructions. PCR of genomic DNA specifically targeted and amplified 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*) of *B. burgdorferi* s.l., as described previously (Postic et al. 1994) and, similarly, a portion of the *OspA* gene (Morshed et al. 2005).

PCR amplifications were performed using a HotStarTaq Master Mix kit (QIAGEN, Mississauga, ON). A total of 5 µl of extracted DNA was used for a 25 µl reaction. For the 5S-23S (*rrf-rrl*) reaction, primer 1 (CTGCGAGTTCGCGGGAGA) and primer 2 (TCCTAGGCATTCACCATA), both from the supplier (Sigma, Oakville, Ontario), were utilized. The conditions for thermal cycling were as follows: initial denaturation at 95° C for 10 min, followed by 50 amplification cycles, which included a denaturing step at 95° C for 1 min, and an annealing step at 52° C for 1 min, and extension at 72° C for 2 min, then ending with 72° C for a 7 min extension. For the *OspA* reaction, primer 3 (TTCTGACGATCTAGGTCAAA) and primer 4 (GCAGTTAAAGTTCCTTCAAG) were used. The conditions for thermal cycling were similar to *rrf-rrl* reactions but annealing temperature was 55° C.

Amplification was carried out using negative and positive controls for all PCR reactions using sterile water and purified *B. burgdorferi* s.s. type strain B31, respectively.

Gene sequencing

PCR products were purified using QIAquick PCR Purification Kits (QIAGEN, Mississauga, ON). Amplicons from *Borrelia* specific reactions were sequenced for species identification by the dideoxy chain termination method using the same primer sets as described above with the BigDye Terminator Cycle Sequencing kit on GeneAmp PCR System 9700 (Applied Biosystems Co., Foster City, CA). The cycling reactions were cleaned by precipitation with a sodium acetate/ethanol mixture, formamide denatured. The *rrf-rrl* amplicons were sequenced with an ABI Prism 3100/3130 DNA Sequencer (Applied Biosystems Co., Foster City, CA). Cluster analysis of the *rrf-rrl* locus was conducted using SeqMan and MegAlign modules within the Lasergene Sequence Analysis software (DNASTAR Inc., Madison, WI). A cladogram tree of the *Borrelia* sequences including representative sequences of *Borrelia* species downloaded from GenBank (National Center for Biotechnology Information, Bethesda, MD) was generated to determine the taxa relationships by Clustal X analysis using the MegAlign Module of the Lasergene software. The basic local alignment search tool (BLAST) was performed on the *Borrelia* genospecies and taxa to help compare them with known *Borrelia* species and determine the closest isolates in the GenBank nucleotide databases (www.ncbi.nlm.nih.gov/BLAST). The 218-bp *rrf* (5S)-*rrl* (23S) intergenic sequences are listed in the table format and have been deposited in GenBank.

RESULTS

Bird-tick associations

In total, 366 field-collected, ixodid ticks representing 12 species were sampled from 151 infested songbirds (31 species/subspecies) at 16 localities across Canada during a three-year period (15 April 2004 to 23 October 2006) (Table 1, Figure 1). *Ixodes* species included: *I. auritulus* (ten females, 121 nymphs, 96 larvae); *I. brunneus* (two females); the rabbit-associated tick, *I. dentatus* Marx (two larvae); the

Table 1. Occurrence of ticks on infested songbirds and presence of *B. burgdorferi* s.l. in ticks in Canada, 2004-2006.

Bird species/subspecies	No. birds	No. ticks on birds	No. birds with *B. burgdorferi*-positive ticks
Song Sparrow, *Melospiza melodia* (Wilson)	31	112	6
Swainson's Thrush, *Catharus ustulatus* (Nuttall)	19	38	3
Lincoln's Sparrow, *Melospiza lincolnii* (Audubon)	14	32	1
Common Yellowthroat, *Geothlypis trichas* (L.)	14	16	3
White-throated Sparrow, *Zonotrichia albicollis* (Gmelin)	10	29	1
Spotted Towhee, *Pipilo maculates* Swainson	9	13	0
White-crowned Sparrow, *Zonotrichia leucophrys* (Forster)	6	6	0
Golden-crowned Sparrow, *Zonotrichia atricapilla* (Pallas)	5	11	1
Magnolia Warbler, *Dendroica magnolia* (Wilson)	5	7	0
Fox Sparrow, *Passerella iliaca* (Merrem)	4	16	0
Winter Wren, *Troglodytes troglodytes* (L.)	4	11	1
Oregon Junco[a], *Junco hyemalis oreganus* (Townsend)	4	11	1
Hermit Thrush, *Catharus guttatus* (Pallas)	3	26	0
Swamp Sparrow, *Melospiza georgiana* (Latham)	3	5	0
America Robin, *Turdus migratorius* L.	2	3	2
Purple Finch, *Carpdacus purpureus* (Gmelin)	2	2	0
MacGillivray's Warbler, *Oporornis tolmiei* (Townsend)	2	2	0
Chipping Sparrow, *Spizella passerina* (Bechstein)	1	2	0
Savannah Sparrow, *Passerculus sandwichensis* (Gmelin)	1	1	0
Brown Thrasher, *Toxostoma rufum* L.	1	3	0
Gray Catbird, *Dumetella carolinensis* (L.)	1	1	0
Philadelphia Vireo, *Vireo philadelphicus* (Cassin)	1	1	0
Red-eyed Vireo, *Vireo olivaceus* (L.)	1	1	0
Gray-cheeked Thrush, *Catharus minimus* (Lafresnaye)	1	1	0
Ovenbird, *Seiurus aurocapillus* (L.)	1	1	0
Dark-eyed Junco, *Junco hyemalis hyemalis* (L.)	1	9	0
Black-and-white Warbler, *Mniotilta varia* (L.)	1	1	0
Wilson's Warbler, *Wilsonia pusilla* (Wilson)	1	1	0
Yellow Warbler, *Dendroica petechia* L.	1	2	0
Yellow-rumped Warbler, *Dendroica coronata* (L.)	1	1	0
House Wren, *Troglodytes aedon* Viellot	1	1	0
Total	151	366	19

[a] The Oregon Junco, a subspecies of the Dark-eyed Junco, predominantly inhabits western Canada.

mouse tick, *I. muris* Bishopp & Smith (four females, three nymphs); *I. pacificus* (five nymphs, 16 larvae); *I. scapularis* (22 nymphs, seven larvae). *Amblyomma* species ticks; namely, the lone star tick, *A. americanum* L. (one nymph); *A. imitator* Kohls (one nymph); *A. longirostre* Koch (two larvae); the Gulf Coast tick, *A. maculatum* Koch (two larvae); and *A. sabanerae* Stoll (one nymph) are listed in Table 2. Rabbit ticks, *Haemaphysalis leporispalustris* Packard (15 nymphs, 55 larvae) were collected coast to coast.

Mean intensity and coinfestation of *Ixodes* ticks

The mean intensity (average number of ticks per infested bird) for all Canadian sites was 2.4 (range, 1-19). The highest infestation occurred on a White-throated Sparrow, which had 19 *H. leporispalustris* (three nymphs, 16 larvae) collected on 24 September 2004 at Revelstoke, B.C. Ticks were most frequently found on ground-foraging and shrub-frequenting songbirds. Along coastal B.C, the mean intensity of *I. auritulus* on infested songbirds was 2.6 (range: 1-10); the highest seasonal mean intensity was 3.5, which occurred during late fall migration (Table 3). Co-infestations of two different tick species were observed on six individual birds (Table 4). Both subadult stages of *I. pacificus* (two nymphs, eight larvae) were found simultaneously co-feeding on a Song Sparrow on 14 August 2004 at Rocky Point, Vancouver Island, B.C. denoting an established population of this tick species in the vicinity.

Seasonal occurrence of *Ixodes* ticks in British Columbia

All three active stages of *I. auritulus* were collected throughout the bird banding season (April-October) along south-coastal B.C.: larvae were recorded from 21 April to 15 October; nymphs from 22 April to 15 October; and females from 16 April to 12 October. Fully engorged *I. auritulus* nymphs from three songbirds were reared to males with a mean molt interval of 45 days (range: 44 to 48 days). For *I. pacificus*, larvae exhibited a temporal pattern from 29 May to 14 August, and nymphs from 28 May to 20 September. New host records of bird parasitism for *I. auritulus* in Canada include: Lincoln's Sparrow, Purple Finch, MacGillivary's Warbler, Spotted Towhee, White-crowned Sparrow, Fox Sparrow, Yellow Warbler, and Oregon Junco. In all, 98 (43%) of 227 *I. auritulus* were reported during mid-autumn migration (1 to 30 September), which underlines the potential for these ticks to be widely dispersed. *Ixodes pacificus* is reported for the first time on Song Sparrow, Spotted Towhee, and Winter Wren in Canada.

Spirochete detection

Using PCR amplification of the *rrf-rrl* spacer, 11.4% (19/167) of tested ticks were positive for *B. burgdorferi* s.l. (Table 5) and, likewise, confirmed positive by the *OspA* gene. *Borrelia burgdorferi* s.l. DNA was not found in any of the negative controls. Cluster analysis of the *rrf-rrl* region of the 19 isolates revealed four genotypes (*B. burgdorferi* s.s. [n=5], BC genotype 1 [n=3], BC genotype 2 [n=6], and BC genotype 3 [n=5]), which are illustrated (Figures 2 and 3; Table 6). When principal tick vectors (*I. pacificus* and *I. scapularis*) were combined, 8.8% (3/34) were infected with *B. burgdorferi* s.l. Lyme disease spirochetes were detected in engorged *Ixodes* larvae collected from American Robin, Common Yellowthroat, Golden-crowned Sparrow, Song Sparrow, and Swainson's Thrush (Table 6).

Table 2. Occurrence of *Amblyomma* spp. ticks on individual migratory songbirds in southern Canada, by province.

Bird species	Site[b] no.	Date ticks collected	*Amblyomma* ticks[a]					
			americanum	*imitator*	*longirostre*		*maculatum*	*sabanerae*
			N	N	L	N	L	N
			Alberta					
Swainson's Thrush	10	12-V-2004	1	0	0	0	0	0
			Ontario					
Magnolia Warbler	5	17-V-2004	0	0	1	0	0	0
Magnolia Warbler	5	17-V-2004	0	0	1	0	0	0
Gray Catbird	5	25-V-2004	0	1	0	0	0	0
Red-eyed Vireo	5	26-V-2004	0	0	0	1	0	0
Philadelphia Vireo	5	26-V-2004	0	0	0	0	1	0
Magnolia Warbler	5	27-V-2004	0	0	0	0	1	0
Black-and-white-Warbler	5	26-V-2004	0	0	0	0	0	1
Total birds: 8 (6 species)			1	1	2	1	2	1

[a] L, larva(e); N, nymph(s). [b] See Figure 1 for site locations.

Table 3. Seasonal intensity and *B. burgdorferi* s.l. infection for *I. auritulus* collected from songbirds in the Georgia Basin, British Columbia, Canada, 2004.

Songbird activity period[b]	*I. auritulus* Larva(e)	Nymph(s)	Female(s)	No. birds infested	Mean intensity of ticks on infested birds	Pos.[a] ticks/ no. of birds infested (%)
Spring migration (16 April to 30 May)	14	3	3	10	2.1	3/10 (30)
Nesting-fledgling (1 June to 20 July)	8	5	0	6	2.2	1/6 (10)
Early autumn migration (21 July to 31 Aug.)	2	23	1	15	1.7	3/15 (20)
Mid-autumn migration (1 Sept. to 30 Sept.)	28	66	4	37	2.6	4/37 (11)
Late fall migration (1 Oct. to 15 Oct.)	44	24	2	20	3.5	5/20 (25)
Total	96	121	10	88	2.6	16/88 (18)

[a]*Bbsl*-pos., *B. burgdorferi* s.l. positive. [b] Songbird activity periods were based on general breeding, nesting, and migration patterns observed during the local bird banding season.

Table 4. Coinfestations of different *Ixodes* spp. collected from individual songbirds in Canada.

Bird species	Site no.[b]	No. of birds exam.[c]	Date ticks collected	*Ixodes* ticks[a] *I. auritulus* L	N	F	*I. dentatus* L	N	*I. pacificus* L	N	*I. scapularis* L	N
Song Sparrow	13	31	03-VI-2004	3	4	0	0	0	1	0	0	0
Song Sparrow	13	31	21-VI-2004	0	1	0	0	0	1	0	0	0
Song Sparrow	16	31	14-VIII-2004	0	4	0	0	0	8	2	0	0
Swainson's Thrush	16	19	05-IX-2004	0	7	0	0	0	0	1	0	0
Song Sparrow	14	31	20-IX-2004	2	1	0	0	0	0	1	0	0
Swamp Sparrow	7	3	07-V-2005	0	0	0	1	0	0	0	0	2

[a] L, larva(e); N, nymph(s); F, female(s). [b] See Figure 1 for site locations. [c] Exam., examined.

DISCUSSION

Our Canadian, bird-tick-spirochete study reveals four *B. burgdorferi* s.l.-infected, tick species: *I. auritulus*, *I. muris*, *I. pacificus*, and *I. scapularis*. Our results corroborate the previous findings of Morshed et al. (2005) who showed that Passeriformes are involved in the enzootic cycle of *B. burgdorferi* s.l. in B.C. In coastal B.C. environments, four different *B. burgdorferi* s.l. genotypes were detected in ticks collected from songbirds. Antigens of these local genotypes will be supportive for serological testing of Lyme disease patients. During spring migration, several species of bird-transported ticks are carried to the Canadian North during rapid migratory flight. This is the first time that we have encountered three genera of ixodid ticks on songbirds in northwestern Canada and, nationally, four different avifaunal *Ixodes* species were infected with *B. burgdorferi* s.l.

Ticks in the Canadian north

Our study provides the first records of ticks in the Yukon, which is north of 60° latitude. Three species of ticks (*I. brunneus*, *I. muris*, and *H. leporispalustris*) were collected from neotropical migrant Passeriformes at Albert Creek Bird Observatory (Site 11) near Watson Lake, Yukon. Each spring, migratory songbirds fly northward to breeding and nesting sites in this insect-rich, continent-wide boreal forest, which spans north-central Canada, including southern Yukon. Specifically, an engorged *I. brunneus* female was collected from a Gray-cheeked Thrush on 25 May 2004; the collection of this extra-limital tick species is unprecedented in this northern region and denotes the first recovery west of Manitoba. In total, five *I. muris* were collected at the Yukon site. Specifically, an engorged *I. muris* female was collected from a Common Yellowthroat on 25 July 2005. Likewise, a fully engorged *I. muris* nymph was retrieved from a Swamp Sparrow on 26 July 2005 and, similarly, a partially engorged *I. muris* female was collected from a Swainson's Thrush on 2

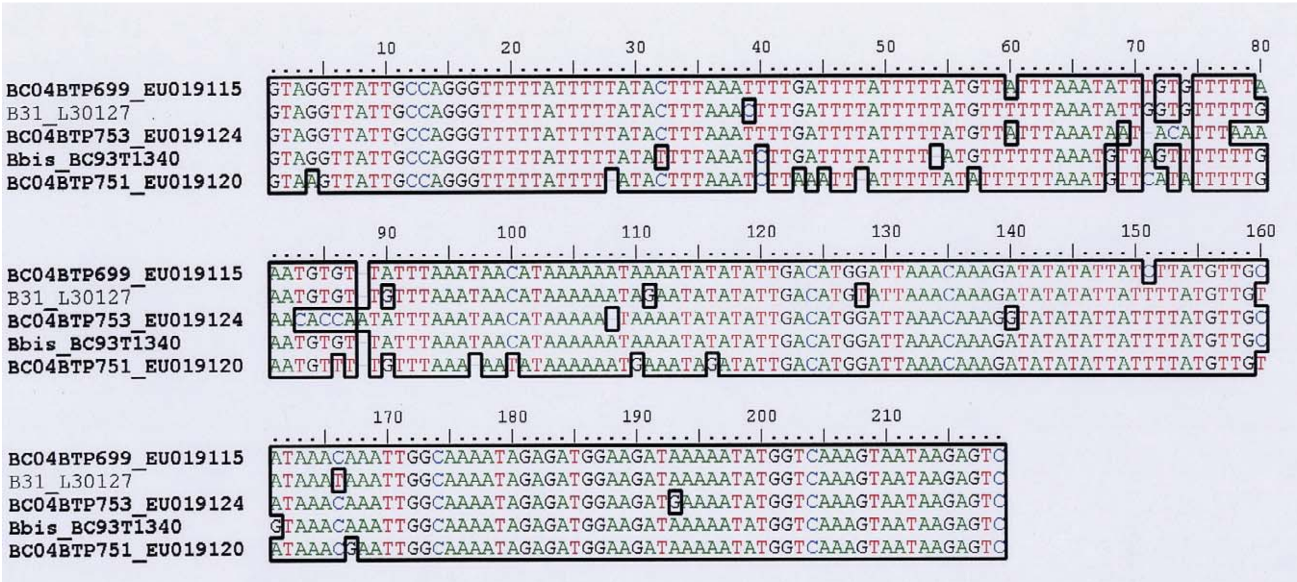

Figure 2. Nucleotide sequence alignment of 218-bp fragments of *rrf* (5S)-*rrl* (23S) intergenic spacer amplicons of B.C. isolate BC04BTP699, isolate BC04BTP751, isolate BC04BTP753, *B. bissettii* isolate BC93T1340 (GenBank accession no. AY077830, from Squamish, B.C.), and reference *B. burgdorferi* s.s. strain B31 (L30127). Mismatches are boxed and highlighted in boldface type. Gaps consist of 1-bp deletions. Canadian-based isolates for this study are in boldface type.

August 2005. The following year, individual *I. muris* nymphs were recovered from a Wilson's Warbler and a Swainson's Thrush on 20 May 2006 and 27 May 2006, respectively. The occurrence of an *I. muris* nymph and a female on recaptured songbirds at this Yukon locality during the nesting-fledgling period suggests that this tick species is established in the area. Likewise, a replete *H. leporispalustris* larva was collected from a Swainson's Thrush on 16 August 2004 and, after holding at 95% humidity, it molted to a nymph in 29 days. Since the larval *H. leporispalustris* was on a neotropical migrant, which only stays ≈100 days to breed and nest, this collection indicates that this tick species is established in the nearby locale. Biogeographically, *H. leporispalustris* has country-wide distribution, including Newfoundland and Labrador, Nova Scotia, Ontario, Alberta, British Columbia, and Yukon. Not only are these three bird-transported tick species reported north of the 60th parallel for the first time, they constitute the northernmost records for any ixodid ticks in Canada.

***Amblyomma* ticks on songbirds**

Our study provides the most northern record of *A. americanum* in North America (Table 2). It was collected from a Swainson's Thrush at the town of Slave Lake, Alberta on May 12, 2004. This engorged nymph molted to a male in 44 days. *Amblyomma* species ticks are not indigenous to Canada and are imported annually by songbirds during spring migration. The number of *Amblyomma* ticks was scant because another concurrent ectoparasite study (2005 to 2006) secured them in eastern Canada. However, medical practitioners have consistently collected *A. americanum* and *A. maculatum* from dogs, cats, and humans, which have no history of travel, during mid-summer (Morshed et al. 2006, J.D.S. unpublished data); some of these hosts become clinically ill after tick bites and, when treated with antimicrobials, febrile symptoms resolve. In particular, *A. americanum* can harbor: *Borrelia lonestari* (etiological agent of southern tick-associated rash illness [STARI] or Masters disease), *Ehrlichia chaffeensis* (human monocytic ehrlichiosis), *Ehrlichia ewingii* (granulocyctic ehrlichiosis), *Francisella tularensis* (tularemia), and *Rickettsia amblyommii* (spotted fever group rickettsia) (Stromdahl et al. 2008). Masters et al. (1998) reported rashes after human *A. americanum* bites that were visibly similar to or indistinguishable from *Ixodes*-associated erythema migrans of Lyme disease, and patients were typically seronegative for antibodies to *B. burgdorferi* s.l. It is reasonable to speculate that, wherever *I. scapularis* and *A. americanum* co-occur, some proportion of reported erythema migrans-diagnosed Lyme disease cases might be the result of *B. lonestari* and/or *R. amblyommii*-infected *A. americanum* imported by migratory passerines. The avian dispersal of *A. americanum* east of the Rocky Mountains has clinical and public health ramifications for patients and medical professionals.

An *A. imitator* was collected from a Gray Catbird at Long Point, Ontario; it is reported for the first time on a bird in Canada and is the first record of this tick species in Canada. This neotropical tick principally ranges from southern Texas southward through Mexico into Central America, and parasitizes various species of birds and mammals (Keirans and Durden 1998). Another neotropical tick, *Amblyomma longirostre*, which parasitizes canopy songbirds and arboreal porcupines, is indigenous from Panama to southern Brazil (Labruna et al. 2007). Passerines transport these slow-feeding, subadult ticks to southern Canada, and fly 500 km/day or more during spring migratory flight, especially when aided by south winds and warm temperatures.

Table 5. Ticks collected from songbirds across Canada tested for *B. burgdorferi* sensu lato, 2004-2006.

Bird species	Tick/pools tested		
	Tick species[a]	No. of ticks	Bbsl-pos.[b]
Song Sparrow	Hlp	2	0
	Iar	34	5
	Ipc	10	1
	Isc	1	0
Swainson's Thrush	Iar	13	3
	Isc	3	0
Lincoln's Sparrow	Hlp	1	0
	Iar	20	1
	Isc	1	0
Common Yellowthroat	Iar	7	1
	Isc	7	2
White-throated Sparrow	Hlp	3	0
	Iar	1	0
	Idn	1	0
	Imu	1	1
	Isc	2	0
Spotted Towhee	Iar	10	0
	Ipc	1	0
White-crowned Sparrow	Iar	5	0
	Ibr	1	0
Golden-crowned Sparrow	Iar	5	1
Magnolia Warbler	Isc	2	0
Fox Sparrow	Iar	8	0
Winter Wren	Iar	5	1
	Ipc	1	0
Oregon Junco[c]	Iar	5	1
Hermit Thrush	Iar	2	0
Swamp Sparrow	Idn	1	0
	Isc	3	0
American Robin	Iar	3	2
Purple Finch	Iar	1	0
MacGillvray's Warbler	Iar	2	0
Chipping Sparrow	Isc	2	0
Brown Thrasher	Isc	1	0
Yellow Warbler	Iar	1	0
House Wren	Imu	1	0
Total bird species: 21		167	19

[a] Tick species: Hlp, *Haemaphysalis leporispalustris*; Iar, *Ixodes aurtulus*; Ibr, *I. brunneus*; Idn, *I. dentatus*, Imu, *I. muris*; Ipc, *I. pacificus*; and Isc, *I. scapularis*. Unlisted ticks are voucher specimens or are held for other tick studies. [b] Bbsl pos., *Borrelia burgdorferi* sensu lato positive. [c] Oregon Junco, a western subspecies of Dark-eye Junco.

***Borrelia burgdorferi* s.s.**

When the 19 *rrf-rrl* spacer amplicons were analyzed using the MegAlign Module, five isolates clustered into the clade associated with *B. burgdorferi* s.s. strain B31 (L30127). Two isolates were from coastal B.C. while three were from eastern Canada (Figure 3). Interestingly, the West Coast isolate BC04BTP711 had no recognizable sequence divergence (percent sequence variation; smaller percent indicates closer distance) from the East Coast isolate (NS06BTP916). This close similarity suggests transcontinental movement of Lyme disease spirochetes in vector ticks. Some passerines partially or completely cross the continent diagonally (southeast to northwest) during migration. When ticks are carried to southern climes and molt, they can later reattach to another songbird, and be transported northward in a different flight pattern to the other side of North America. Not only has *B. burgdorferi* s.s. been detected across North America, it has been detected in *I. ricinus* subadults on songbirds in Europe and western Russia (Alekseev et al. 2001, Gorelova et al. 2001, Poupon et al. 2006); transatlantic movement of this genospecies, via bird-transported ticks and wild birds as reservoirs, is therefore plausible.

BC genotype 1

Three *B. burgdorferi* s.l. strains clustered together in BC genotype 1, the most divergent subcategory in our study. Isolate BC04BTP751 had the closest genetic link to reference strain *B. valaisiana* (L30133) with 6.4% sequence divergence. Likewise, isolate BC04BTP751 had a 7.4% sequence divergence with *B. valaisiana* strains (L30134, AB022127) and *B. garinii* (AB035964). In nature, *B. valaisiana* is widely distributed from Ireland to Far East Asia (Kirstein et al. 1997, Masuzawa et al. 2000).

The Winter Wren, which was parasitized by an *I. auritulus* nymph harboring isolate BC04BTP751, has circumpolar distribution in Eurasia and North America. During windstorms and migratory flight, Winter Wrens may traverse the Bering Strait with *B. burgdorferi* s.l.-infected ticks and, using this ornithological connecting link, introduce new genotypes to the Americas. Several studies report *B. burgdorferi* s.l. in *I. ricinus* collected from Winter Wrens in Eurasia (Alekseev et al. 2001, Mannelli et al. 2005), and *B. valaisiana* was one of the most prominent or frequently occurring genospecies (Poupon et al. 2006, Taragel'ova et al. 2008). Ecologically, Winter Wrens migrate and breed along the Pacific coast where *I. auritulus* is indigenous (Durden and Keirans 1996). One B.C. isolate (BC04BTP672) was obtained 14 km from two other isolates (i.e., BC04BTP732, BC04BTP751), which indicates a continuum of this *Borrelia* clade within the Georgia Basin bioregion in southwestern B.C.

BC genotype 2

Six isolates grouped in BC genotype 2. Isolate BC04BTP753 had the closest genetic link to isolate BC02T1398 (BC genotype 3 clade) with 8.3% sequence divergence. Likewise, isolate BC04BTP753 had a 9.4%

Table 6. *Borrelia burgdorferi* sensu lato infections in *Ixodes* spp. ticks feeding on different bird species/subspecies in Canada, by genotype, 2004-2006.

Genotypes	Site, prov.[d]	Ticks collected dd/mm/yy	*Ixodes* ticks[a] *Iar* L	*Iar* N	*Iar* F	*Imu* F	*Ipc* L	*Ipc* N	*Isc* L	*Isc* N	Isolate[c] no.	GenB[b] no. EU01
BC genotype 1												
American Robin	13, BC	06-VI-2004	1[e]	0	0	0	0	0	0	0	BC04BTP672	9112
Winter Wren	14, BC	05-X-2004	3	1[e]	0	0	0	0	0	0	BC04BTP751[c]	9120
Song Sparrow	14, BC	06-X-2004	1[e]	2	0	0	0	0	0	0	BC04BTP732	9121
BC genotype 2												
American Robin	14, BC	28-V-2004	0	1[e]	0	0	0	0	0	0	BC04BTP660	19111
Swainson's Thrush	14, BC	28-VII-2004	0	0	1[e]	0	0	0	0	0	BC04BTP686	9113
Common Yellowthroat	14, BC	20-VIII-2004	0	1[e]	0	0	0	0	0	0	BC04BTP697	9114
Song Sparrow	14, BC	24-IX-2004	0	4	1[e]	0	0	0	0	0	BC04BTP726	9119
Song Sparrow	14, BC	10-X-2004	1	2[f]	0	0	0	0	0	0	BC04BTP753[c]	9124
Oregon Junco	14, BC	10-X-2004	7[g]	1[g]	0	0	0	0	0	0	BC04BTP734	9122
C genotype 3												
Swainson's Thrush	14, BC	11-V-2004	2[f]	0	0	0	0	0	0	0	BC04BTP658	9110
Swainson's Thrush	14, BC	28-VIII-2004	0	2[h]	0	0	0	0	0	0	BC04BTP699[c]	9115
Song Sparrow	14, BC	05-IX-2004	0	6[f]	0	0	0	0	0	0	BC04BTP707	9116
Song Sparrow	14, BC	20-IX-2004	2	1	0	0	0	1[e]	0	0	BC04BTP722[c]	9118
Lincoln Sparrow	14, BC	11-X-2004	1	3[f]	0	0	0	0	0	0	BC04BTP752	9123
B. burgdorferi s.s.												
Golden-crowned Sparrow	14, BC	21-IV-2004	2[f]	0	0	0	0	0	0	0	BC04BTP645	9109
Song Sparrow	14, BC	07-IX-2004	3[f]	3	0	0	0	0	0	0	BC04BTP711	9117
Common Yellowthroat	3, NB	22-V-2004	0	0	0	0	0	0	0	1[e]	NB04BTP655	9125
White-throat Sparrow	2, NS	21-IX-2006	0	0	0	1[e]	0	0	0	0	NS06BTP916	9126
Common Yellowthroat	4, ON	10-X-2006	0	0	0	0	0	0	1[e]	0	ON06BTP917	9127

[a] L, larva(e); N, nymph(s); F, female(s). Tick species: *Iar*, *Ixodes auritulus*; *Imu*, *I. muris*; *Ipc*, *I. pacificus*; *Isc*, *I. scapularis*. [b] GenB no., GenBank accession number; EU01 is the prefix, and the suffixes are listed in the column below. [c] Live culture. [d] See Fig. 1 for site locations. Provinces: BC, British Columbia; MB, Manitoba; NB, New Brunswick; NS, Nova Scotia; ON, Ontario. [e] Single tick positive. [f] Pool of ticks positive for one developmental life stage. [g] A pool of two development life stages combined was positive. [h] One of two ticks was positive; slightly engorged tick negative, partially engorged tick positive.

sequence divergence with *B. bissettii* (AF230079) and, similarly, 9.9% with *B. bissettii* strain BC93T1340 (AY077830). This B.C. isolate (BC93T1340; Squamish, 1993) was cultured from *I. angustus* larvae attached to a wild-caught deer mouse, *Peromyscus maniculatus* (Wagner), a capable reservoir of *B. bissettii* (Eisen et al. 2003); these spirochetes were described as "*B. burgdorferi*" (Banerjee et al. 1994a) but, using DNA sequencing, have recently been delineated to *B. bissettii*. European bird- and rodent-associated studies reveal that *B. bissettii* has no apparent association with songbirds (Kurtenach et al. 2002, Hanincová et al. 2003).

BC genotype 3

Five *B. burgdorferi* s.l. isolates formed a new branch, BC genotype 3, which falls between the *B. bissettii* and *B. burgdorferi* s.s. clades. These five isolates shared similar sequences with isolates that were previously detected in *I. auritulus* ticks collected on Vancouver Island, B.C. (Morshed et al. 2005). Based on BLAST sequence analysis, isolate BC04BTP699, the initial isolate of this genotype in our study, had 100% sequence similarity to isolate BC02T1398 (AY363396) previously cultured from an *I. auritulus* female removed from an American Robin at Merville, B.C. in 2002. Also, isolate BC04BTP699 had a 4.3% sequence divergence with *B. burgdorferi* s.s. isolates (B31, BC04BTP711) and, similarly, 4.3% with *B. bissettii* strains (AF230079, BC93T1340). Because isolate BC02T1398 was obtained in a previous study, it takes precedence; therefore, the five isolates in our study were designated to BC genotype 3. Using BLAST analysis of the *rrf-rrl* spacer, isolate BC04BTP699 from Wilson Creek, B.C. (Site 14), on

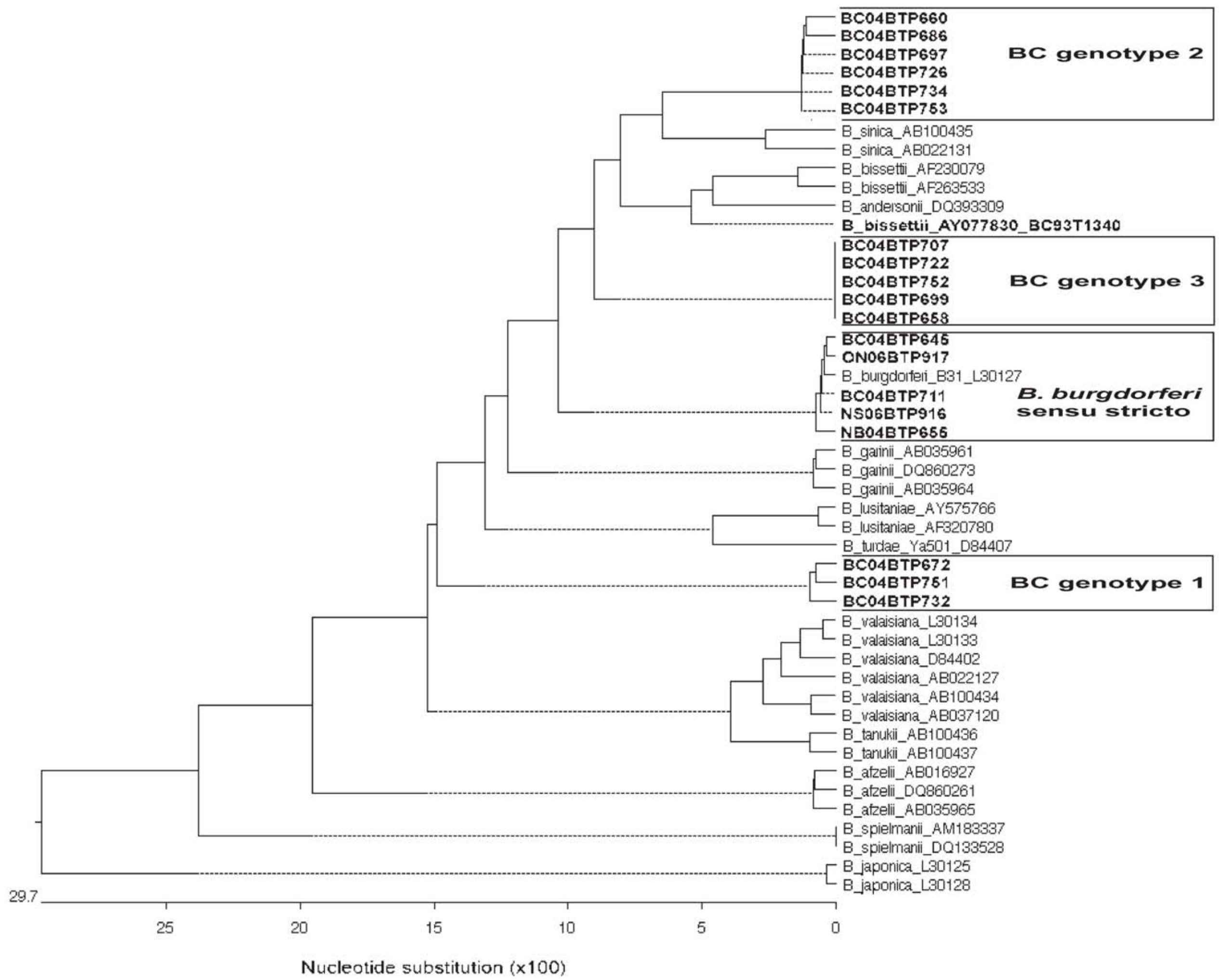

Figure 3. Cladogram of the 218-bp fragments of the *rrf* (5S)-*rrl* (23S) intergenic spacer for *B. burgdorferi* s.l. isolates obtained in this study and reference strains available from the GenBank database using the neighbor-joining algorithm of MegAlign program. Alignment was completed using SeqMan and MegAlign modules within the Lasergene Sequence Analysis software. The Canadian-based *Borrelia* amplicons for this study are in boldface type. The bar represents nucleotide substitutions after conducting 100 replications.

mainland B.C., was highly homologous with the BC02T1398 isolate from Vancouver Island, an overwater flight of 101 km. Interestingly, three other strains, which were previously detected in *I. auritulus* from Vancouver Island (Morshed et al. 2005), are homologues of BC02T1398. In the present study, isolate BC04BTP722, a BC genotype 3 strain, was cultured from an *I. pacificus* nymph collected from a Song Sparrow at Wilson Creek, B.C.; it represents the first isolate from an *I. pacificus* collected from a songbird in Canada.

Cluster analysis of the *rrf-rrl* spacer gene

Using sequence analysis of the *rrf-rrl* intergenic spacers, 19 *B. burgdorferi* s.l. strains segregated into four distinct lineages (Figure 3). The three novel genotypes differ significantly from *B. burgdorferi* s.s. Although the nucleotide sequence for the *rrf-rrl* spacer region is not highly conserved, it provides a helpful tool to determine strain diversity between species (Richter et al. 2006, Postic et al. 2007). Since the *rrf-rrl* spacer alone has limitations for establishing *B. burgdorferi* s.l. genospecies nomenclature, further multilocus sequence analysis using a combination of highly conserved genes (i.e., *fla*, *OspA*) is needed to taxonomically delineate and genomically classify the new genotypes (BC genotype 1, BC genotype 2, BC genotype 3) within the *B. burgdorferi* s.l. complex.

***B. burgdorferi* s.l. in 3-tick cycle in British Columbia**

Along south-coastal B.C., *I. angustus*, *I. auritulus*, and *I. pacificus* coexist together within certain ecosystems. These three tick species could either be involved in shared, intertwining cycles or separate, contiguous enzootic cycles. Banerjee et al. (1994a, 1994b) reported "*B. burgdorferi*"-infected *I. angustus* and *I. pacificus* on *P. maniculatus* mice and humans. Not only do deer mice act as common tick hosts in temperate Nearctic areas, they are competent carriers for Lyme disease spirochetes (Eisen et al. 2003). Although *I. angustus* does not parasitize songbirds, it occasionally parasitizes dogs, cats, and people (Damrow et al. 1989, Peavey et al. 2000). At one locality (Site 14), four different genotypes of *B. burgdorferi* s.l. were detected in *I.*

auritulus collected from passerines. Although not a human-biting tick, *I. auritulus* plays an enzootic role as a vector of *B. burgdorferi* s.l. On five separate occasions, *I. auritulus* and *I. pacificus* simultaneously infested passerines. These coinfestations show the potential for tick-host-tick cross-transmission of *B. burgdorferi* s.l. between two different tick species and, similarly, co-transmission within a single tick species. *Ixodes pacificus* has a wide diversity of vertebrate hosts and can act as an interconnecting enzootic link for Lyme disease spirochetes between a multitude of hosts, including a bridge vector to humans. Collectively, *I. angustus*, *I. auritulus*, and *I. pacificus* overlap in a web-like network in certain wildlife communities along coastal B.C., which supports the hypothesis that these three sympatric ticks are interconnected in a three-tick enzootic transmission cycle complex of *B. burgdorferi* s.l.

Cross-border importation of ticks on songbirds during spring migration

This study provides new data on the transborder influx of avian-associated ticks into Canada via migratory songbirds. Scott et al. (2001) reported *B. burgdorferi*-positive *I. scapularis* on passerines, which were collected on Portage Island, an island off the south coast of Nova Scotia; this non-endemic island is one of the first contact points where birds would make landfall in Atlantic Canada. In the present study, a fully engorged *B. burgdorferi* s.s.-positive *I. scapularis* nymph was collected during peak northward spring migration from a Common Yellowthroat, a neotropical migrant, at St. Andrews, New Brunswick, on the north bank of the St. Croix River, a non-endemic area, just north of the U.S.-Canada border; this collection confirms that northward-migrating songbirds introduce spirochete-infected ticks into Canada.

Of epidemiological significance, it is claimed that 2 to 4.5 billion songbirds migrate north into Canada each spring (Ogden et al. 2008). Based on tick counts of infested songbirds by experienced examiners, we estimate that 45 million *I. scapularis* immatures are imported into Canada during spring migration, and an additional 15 million are acquired from established populations in southern Canada and dispersed locally or transported to northern latitudes.

Tick parasitism of songbirds during southward autumn migration

We provide new evidence of *B. burgdorferi* s.l.-positive ticks on songbirds in Canada during southward autumn migration. By late August, the majority of boreal-nesting passerines and their progeny fly south to complete their biannual migration from the Canadian North to wintering ranges in the southern United States, or Central and South America (Anderson et al. 1990, Hyland et al. 2000, Durden et al. 2001). Typically, *H. leporispalustris* (larvae and nymphs) is the dominant tick species on autumn-migrating songbirds in northeastern and central North America (Scott et al. 2001). In Atlantic Canada, a non-engorged *I. scapularis* nymph was collected from a Song Sparrow on 11 September 2006 at Amherst Point, Nova Scotia, during autumn migration. Similarly, a larval *I. scapularis* was collected during late fall migration from a juvenile Common Yellowthroat, which collided with a mirror-glassed highrise in Toronto, Ontario, and *B. burgdorferi* s.s. spirochetes were detected in this tick; this is the first detection of *B. burgdorferi* in an *I. scapularis* larva collected from a bird in Canada. Since transovarial transmission of *B. burgdorferi* s.s. rarely occurs in wild-caught *I. scapularis* larvae (Piesman et al. 1986, Patrican 1997), we hypothesize that the maternal parent was spirochetemic and transmitted infection to the embryonic chick. After departing the nest, this juvenile Common Yellowthroat transmitted spirochetes to the *I. scapularis* larva. The collection of a partially engorged *I. scapularis* nymph from a Swainson's Thrush during autumn migration at the south tip of Pelee Island, Ontario, the southernmost populated point in Canada, reflects southbound ticks destined for the U.S.

Noteworthy is the collection of a partially engorged *I. muris* female on a White-throated Sparrow during autumn migration at Amherst Point, Nova Scotia and, to our knowledge, the first record of a *B. burgdorferi* s.l.-infected *I. muris* on an avian host anywhere; it is also the first report of this tick species on a songbird in this Maritime province. In Maine, Lyme disease spirochetes have been detected in an *I. muris* female removed from a three-year-old child (Dolan et al. 2000). A partially engorged *I. muris* female was also collected in September 2005 from a House Wren at Toronto, Ontario, which underpins the fact that this tick species is also carried southward during autumn migration.

On the Pacific coast, all post-embryonic stages of *I. auritulus* were collected during autumn migration from several resident or semi-migratory songbirds. The collection of four co-feeding *I. auritulus* larvae on 11 September 2004 from a Yellow Warbler, which winters from the southwestern United States to Peru and Bolivia, reveals the potential for southward movement of these ixodid ticks. Undoubtedly, southbound passerines carry *Ixodes* ticks, and some of these bird-transported ticks are infected with Lyme disease spirochetes.

Songbirds as reservoir hosts

We detected *B. burgdorferi* s.l. in four different tick species; namely, *I. auritulus*, *I. muris*, *I. pacificus*, and *I. scapularis* collected from songbirds. Consistent with other studies, we report *B. burgdorferi* s.l.-infected *Ixodes* larvae on songbirds; namely, Swainson's Thrush (Stafford et al. 1995), American Robin (Rand et al. 1998), Song Sparrow (Rand et al. 1998), and Common Yellowthroat (Rand et al. 1998, Weisbrod and Johnson 1989). Relevant to our study, *B. burgdorferi* s.l. has been detected in passerine candidates for reservoir competency: the skin (biopsy) of a Swainson's Thrush (Durden et al. 2001) and the blood (smear) of a Golden-crowned Sparrow (Wright et al. 2000). To our knowledge, we report the first *B. burgdorferi* s.l.-positive *Ixodes* larvae on a Golden-crowned Sparrow. However, to establish reservoir competency of a bird species, essential criteria are needed, as described for American Robins (Richter et al. 2000). In Europe, reservoir competency was also shown for the Blackbird, *Turdus merula* L. using *I. ricinus* larvae (Humair et al. 1998). In our study, six different individual Song Sparrows were parasitized by *B. burgdorferi* s.l.-positive *I. auritulus* (females, nymphs, larvae) and, in two incidences, only larvae were infected. Notably, four different genotypes of *B. burgdorferi* s.l. were detected in *I. auritulus* collected from Song Sparrows. Furthermore, McLean et al. (1993) isolated *B. burgdorferi* s.l from the blood of a Song Sparrow. Likewise, Lyme disease spirochetes have been cultured from blood samples of an American Robin and a Common Yellowthroat (Anderson and Magarelli 1984, Hanincová et al. 2003). The parasitism of *B. burgdorferi*-infected larvae on these five passerine species is suggestive that some, if not all, of these host avian hosts are competent reservoirs.

Risk of tick-associated diseases to humans and domestic animals

Previous studies in central and eastern Canada reveal that 12-13% of *I. scapularis* collected from mammalian hosts, including humans, are infected with *B. burgdorferi* s.l. (Morshed et al. 2006, Ogden et al. 2006). Subsequently, Ogden et al. (2008) reported an *I. scapularis* infestation prevalence of 0.35% on spring migratory songbirds (2.2% when examined by experienced observers), and 15.4% of *I. scapularis* nymphs were infected with *B. burgdorferi* s.l. Epidemiologically, this infection prevalence is analogous to that of *I. scapularis* adults (12.9%) collected by clinicians and veterinarians in Ontario from mammalian hosts, which had no history of travel (Morshed et al. 2006). The close correlation between bird-feeding *I. scapularis* nymphs and mammalian-derived females points to migratory songbirds as key dispersing agents of *Borrelia*-infected ticks.

In North America, multiple zoonotic microbiota are known to be present in *I. pacificus* and *I. scapularis*, which are carried by songbirds and, subsequently, may be transmitted to people and domestic animals. In particular, diverse pathogens have been reported in *I. scapularis*, including *B. burgdorferi* s.l., *Anaplasma phagocytophilum*, *Babesia* spp. (e.g., *B. microti*), *Mycoplasma* spp. (e.g., *M. fermentans*), *Bartonella* spp. (e.g., *B. henselae*), *Borrelia lonestari*, and deer tick virus (Mixson et al. 2006). Likewise, the relapsing fever group spirochete, *Borrelia miyamotoi* s.l., may be imported into Canada by bird-transported *I. scapularis* from the northeastern U.S. (Ullmann et al. 2005). In California, three human pathogens; namely, *B. burgdorferi* s.l., *A. phagocytophilum,* and *Bartonella* sp., have been detected in *I. pacificus* (Holden et al. 2006). Because there are at least 12 different *Ixodes*- and *Amblyomma*-associated pathogens, which may be introduced into Canada by songbirds during spring migration, health-care practitioners in Canada need to be vigilant of them and include them in their clinical differential diagnoses.

Four different genotypes of Lyme disease spirochetes were revealed in ticks spread by songbirds across Canada, and all of these genotypes were isolated from the Georgia Basin. Liang et al. (2002) found that *B. burgdorferi* s.l. has several defense strategies, including antigenic variation, seclusion in privileged sites, and ability to alter the host immune response. Because *B. burgdorferi* s.l. has a myriad of phenotypic and genetic variations, including atypical and cystic forms, this stealth pathogen can survive and persist in multiple vertebrate hosts (MacDonald 2007, Miklossy et al. 2008). In our study, isolate BC04BTP722 (BC genotype 3) was cultured from an *I. pacificus* nymph and, subsequently, could be transmitted to a human or domestic animal. It is well established that *B. burgdorferi* s.s. is infectious and pathogenic; however, the pathogenicity of the three novel B.C. genotypes is unknown. Biomedical studies, which include clinical histories, serological response, and virulence, are needed for patients bitten by *Ixodes* ticks in B.C. Importantly, as noted for Scotland, the use of local *B. burgdorferi* s.l. strains significantly enhances the accuracy and predictive value of Lyme disease serology (Mavin et al. 2009). Thus, all known B.C. genotypes/genospecies should be incorporated into laboratory testing in far-western North America.

Our findings explain the sporadic incidence of bird-derived ticks and tick-borne illnesses, including Lyme disease, outside the known or expected range. Songbirds import *B. burgdorferi* s.l.-infected ticks across the U.S.-Canada border during spring migration, and provide wide-ranging dispersal of bird-feeding ticks at least as far north as Yukon. Our study elucidates the fact that at least four passerine-associated *Ixodes* species (i.e., *I. auritulus*, *I. pacificus*, *I. muris*, and *I. scapularis*) harbor Lyme disease spirochetes in Canada. The occurrence of *B. burgdorferi* s.l.-positive tick larvae on the American Robin, Common Yellowthroat, Golden-crown Sparrow, Song Sparrow, and Swainson's Thrush provides substantive evidence that these avian hosts are competent reservoirs. Greater genetic diversity of *B. burgdorferi* s.l. is more apparent in far-western Canada than previously thought. With the inclusion of *B. bissettii*, five different genotypes/genospecies have been detected in B.C. Most importantly, because songbirds widely disperse *B. burgdorferi* s.l.-infected ticks across Canada, people can contract Lyme disease without any exposure at an endemic area.

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