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John D. Scott[a] & Lance A. Durden[b]
[a] Research Division, Lyme Ontario, Fergus, Ontario, Canada
[b] Department of Biology, Georgia Southern University, Statesboro, GA, USA
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New records of the Lyme disease bacterium in ticks collected from songbirds in central and eastern Canada

John D. Scott[a] and Lance A. Durden[b]

[a]*Research Division, Lyme Ontario, Fergus, Ontario, Canada (jkscott@bserv.com);* [b]*Department of Biology, Georgia Southern University, Statesboro, GA, USA (ldurden@georgiasouthern.edu)*

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This study highlights the collection of ticks from wild-caught birds in central and eastern Canada. Using polymerase chain reaction (PCR), 32 (33%) of 98 *Ixodes* species ticks tested were positive for the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt, and Brenner (hereafter *B. burgdorferi*). Immature blacklegged ticks, *Ixodes scapularis* Say, which are competent vectors of *B. burgdorferi*, constituted the predominant *Ixodes* species tick collected from parasitized songbirds; specifically, 31(35%) of 89 *I. scapularis* nymphs were positive for *B. burgdorferi*. Notably, we report the first *B. burgdorferi*-positive *I. scapularis* (nymph) on a migratory passerine in Prince Edward Island; this is the first record of a tick on a bird in this maritime province. First-time records of *B. burgdorferi*-infected, *I. scapularis* nymphs on passerines in northwestern Ontario include rose-breasted grosbeak, *Pheucticus ludovicianus* (Linnaeus), eastern bluebird, *Sialia sialis* (L.), and indigo bunting, *Passerina cyanea* (L.). Similarly, we furnish a new host record in Canada for an *I. scapularis* (nymph) on a worm-eating warbler, *Helmitheras vermivorus* (Gmelin); this engorged nymph tested positive for *B. burgdorferi*. We document the first recorded collection of a *B. burgdorferi*-infected *Ixodes muris* Bishopp and Smith (female) on a songbird in Ontario. We provide the first account of a live culture of *B. burgdorferi* from an *I. scapularis* (nymph) collected from a brown-headed cowbird *Molothrus ater* (Boddaert) in Manitoba. We make available new host records for *I. scapularis* on a clay-coloured sparrow *Spizella pallida* (Swainson) and a Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii* (Nuttall). An *I. scapularis* on a songbird during southward autumn migration in northwestern Ontario is a first record reported. As well, we document the first-ever account of a reptile-associated tick, a nymph of *Amblyomma sabanerae* Stoll, on a veery *Catharus fuscescens* (Stephens) in northwestern Ontario. Migratory songbirds play an integral role in the wide dispersal of *B. burgdorferi*-infected ticks, especially during northward spring migration. Clinicians need to be aware that people can be bitten by *B. burgdorferi*-infected ticks released by songbirds across Canada.

Keywords: songbirds; ticks; *Ixodes scapularis*; Lyme disease; *Borrelia burgdorferi*; host records; parasitism; Canada

Introduction

Migratory birds have the capacity to fly long distances and widely disperse attached ixodid ticks (Ixodida: Ixodidae). During a blood meal, certain ixodid ticks can transmit pathogenic microorganisms, including the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (hereafter *B. burgdorferi*), to suitable hosts (Burgdorfer et al. 1982). During spring migration, neotropical and southern temperate songbirds (Order: Passeriformes) can transport ticks thousands of kilometres to Canada during long-distance flight from winter grounds in Mexico, Caribbean Islands, and Central and South America (Scott et al. 2001, 2010, 2012; Morshed et al. 2005; Ogden et al. 2008; Scott 2015; Scott and Durden 2015a, 2015b). Infested songbirds can initiate new zoonotic foci of ticks in far-off localities, especially when suitable terrestrial hosts are present (Anderson and Magnarelli 1984; Anderson et al. 1990; Smith et al. 1996; Scott et al. 2014). Notably, passerine migrants can fly as much as 950 km/day during migratory flight (Smith et al. 1996). East of the Rocky Mountains, the blacklegged tick, *Ixodes scapularis*, is the predominant vector of *B. burgdorferi* and is heavily implicated in the transmission of Lyme disease spirochetes to people and domestic animals (Morshed et al. 2006).

Prior to this study, 76 host species records for *I. scapularis* on wild birds had been reported (Anderson and Magnarelli 1980; Anderson 1988; Hyland et al. 2000; Scott et al. 2001, 2012; Durden et al. 2001; Scharf 2004; Morshed et al. 2005; Ogden et al. 2008). East of the Rockies, Lyme disease spirochetes have been detected in four *Ixodes* species (*Ixodes affinis, Ixodes dentatus, Ixodes muris*, *Ixodes scapularis*) (Scott et al. 2001, 2010, 2012, Oliver et al. 2003). The first live culture of *B. burgdorferi* collected from a bird in Canada was isolated from an *I. scapularis* (nymph) in Nova Scotia during cross-border, spring migration (Scott et al. 2001). Subsequently, a live culture of *B. burgdorferi* was obtained in Ontario from an *I. scapularis* nymph infesting a songbird on the north shore of Lake Erie (Scott and Durden 2009). *Borrelia burgdorferi* strains collected from bird-derived ticks in this bioregion showed substantial genetic diversity (Scott and Durden 2009). Not only do wild birds transport ticks, these avifauna can transmit pathogens to attached, blood-sucking, ixodid ectoparasites (Anderson et al. 1986; Richter et al. 2000).

Since passerines are highly mobile, we wanted to investigate the migratory range of bird-feeding ticks and

In the text and references, the name *Ixodes dammini* is a junior synonym of *Ixodes scapularis* determine if they may be implicated in Lyme disease epidemiology in unrepresented areas of central and eastern Canada. The aim of our study was to: (1) expand the geographical range of previous host records, (2) document new bird species host associations, and (3) monitor the prevalence of *B. burgdorferi* in songbird-transported *Ixodes* ticks.

Materials and methods

Tick collection

Ticks were collected from wild-caught songbirds during temperate periods (spring, summer, fall) of the year at nine locations from Manitoba to Prince Edward Island (P.E.I.), 2010–2012 (Figure 1). Using mist-nets, the majority of ticks were collected from passerines by bird banders as time permitted. In addition, ticks that were collected from injured birds as a result of automobile strikes and window strikes were also included. Live ticks were placed in round-bottom, 8.5 mL polypropylene tubes (15.7 × 75 mm) with labels documenting background information. A 7-mm hole was drilled in the polyethylene, push-type caps (15.7 mm diameter) for ventilation. Tulle netting was inserted inside the cap to prevent ticks from escaping. Tubes with field-collected, live ticks were then placed in a self-sealing, double-zipper, plastic bag with a slightly moistened paper towel to maintain 90–95% relative humidity. Ticks were sent directly by express mail to the laboratory (JDS) for morphological identification. Taxonomic keys were employed for tick identification (Clifford et al. 1961; Durden and Keirans 1996; Keirans and Durden 1998). Damaged and dead ticks were put directly in 2 mL micro tubes containing 94% ethyl alcohol to preserve *B. burgdorferi* DNA; retrograde and voucher ticks were not tested.

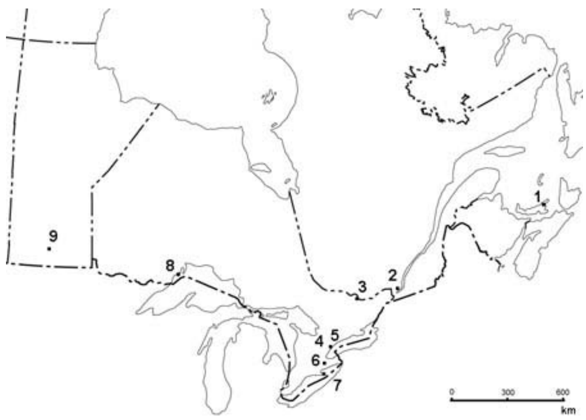

Figure 1. Geographic locations in central and eastern Canada where bird-derived ticks were collected: 1. Brudenell, Prince Edward Island, 46.19ºN, 62.64ºW; 2. McGill Bird Observatory, Ste-Anne-de-Bellevue, Quebec, 45.43ºN, 73.94ºW; 3. Innis Point Bird Observatory, Kanata, Ontario, 45.48ºN, 76.37ºW; 4. Tommy Thompson Park Bird Research Station, Toronto, Ontario, 43.63ºN, 79.33ºW; 5. Fatal Light Awareness Program, Toronto (core area), Ontario, 43.74ºN, 79.37ºW; 6. Ruthven Park National Historic Site Banding Station, Haldimand Bird Observatory, Cayuga, Ontario, 42.97ºN, 79.87ºW; 7. Long Point Bird Observatory, Long Point (Port Rowan), Ontario, 42.52ºN, 80.17ºW; 8. Thunder Cape Bird Observatory, Sibley Peninsula (Thunder Bay), Ontario, 48.42ºN, 88.78ºW; and 9. Delta Marsh Bird Observatory, Delta Marsh (R.R. #2, Portage la Prairie), Manitoba, 50.18ºN, 98.20ºW. Mailing addresses/local urban centres are listed in parentheses.

Spirochete detection

Dead ticks were directly tested using DNA extraction and PCR analysis, whereas live ticks were cultured in Barbour-Stoenner-Kelly (BSK) medium and, subsequently, underwent DNA extraction and PCR amplification. The DNA detection methods have been previously described (Persing et al. 1990a, 1990b; Scott et al. 2013). Although the flagellin gene (*fla*) was used in juxtaposition with the major outer surface protein gene (*OspA*) in Persing et al. (1990b), only the *OspA* gene on the 49-kbp linear plasmid was employed in our study. None of the PCR bands was sent for DNA sequencing. The infection prevalence of *B. burgdorferi* was calculated by dividing the total number of *B. burgdorferi*-positive ticks by the total number of *Ixodes* ticks tested.

Voucher specimens

The larval *Amblyomma longirostre* (Koch) (10-5A28) and nymphal *Amblyomma sabanerae* Stoll (10-5A18) have been deposited in the U.S. National Tick Collection, Georgia Southern University, Statesboro, Georgia (accession numbers RML 124514, and RML 124515, respectively).

Results

A total of 281 ixodid ticks from three genera (*Amblyomma, Haemaphysalis, Ixodes*) were collected from 97 songbirds (30 passerine species) in central and eastern Canada, 2010 to 2012 (Table 1) (Figure 1). These ticks consisted of: 176 *Haemaphysalis leporispalustris* (Packard), 101 *Ixodes* (98 identified, 3 unidentified) (Table 1) and 4 *Amblyomma* (2 identified, 2 unidentified) (Figure 2). Geographically, bird banders and the public from Manitoba to P.E.I. participated in the submission of ticks, especially during spring migration.

Of the *Ixodes* species tested, 32 (33%) of 98 ticks were PCR positive for *B. burgdorferi* (Table 1). Of epidemiological significance, 31 (35%) of 89 *I. scapularis* nymphs were positive for *B. burgdorferi*. For *H. leporispalustris* tested, one nymph was positive for *B. burgdorferi*; this is the first record of a *B. burgdorferi*-infected *Haemaphysalis* sp. on an avian host in Canada. Although southward migrating songbirds were heavily infested with *H. leporispalustris* immatures, we did not screen them all for *B. burgdorferi* because they originated from northern areas which are not noted for sources of Lyme disease spirochetes. Further, none of

Table 1. Detection of *Borrelia burgdorferi* in *Ixodes* species ticks collected from songbirds in central and eastern Canada, 2010–2012.

	brun.	*dent.*	*muris*		*scapularis*		Total positive
Bird species	F	L	L	F	L	N	Number tested (%)
Swamp sparrow *Melospiza geogiana* (Latham)	–	–	–	–	–	1	0/1 (0)
Worm-eating warbler *Helmitheros vermivorus* (Gmelin)	–	–	–	–	–	2	1/2 (50)
Northern Waterthrush *Seiurus noveboracensis* (Gmelin)	–	–	–	–	–	5	0/5 (0)
Common yellowthroat *Geothylypis trichas* (Linnaeus)	–	–	–	–	–	17	5/17 (29)
Swainson's thrush *Catharus ustulatus* (Nuttall)	–	–	–	1	–	14	8/15 (53)
Veery *Catharus fuscescens* (Stephens)	–	1	–	–	1	2	1/4 (25)
Grey-cheeked thrush *Catharus minimus* (Lafresnaye)	–	–	–	–	–	4	0/4 (0)
House wren *Troglodytes aedon* Vieillot	–	–	–	–	–	6	3/6 (50)
White-throated sparrow *Zonotrichia albicollis* (Gmelin)	–	1	–	1	–	5	2/7 (29)
Canada warbler *Wilsonia canadensis* (L.)	–	–	–	–	–	1	0/1 (0)
Savannah sparrow *Passerculus sandwichensis* (Gmelin)	–	–	–	–	–	1	0/1 (0)
Brown-headed cowbird *Molothrus ater* (Boddaert)	–	–	–	–	–	3	3/3 (100)
Mourning warbler *Oporornis philadelphia* (Wilson)	–	–	–	–	–	1	1/1 (100)
Ovenbird *Seiurus aurocapillus* (L.)	–	–	–	–	–	1	0/1 (100)
Black-capped chickadee *Poecile atricapillus* (L.)	–	–	–	–	–	1	0/1 (0)
Lincoln's sparrow *Melospiza lincolnii* (Audubon)	–	–	1	–	–	1	1/2 (50)
Nashville warbler *Vermivora ruficapilla* (Wilson)	–	–	–	1	–	4	0/5 (0)
Yellow warbler *Dendroica petechia* (L.)	–	–	–	–	–	1	1/1 (100)
Indigo bunting *Passerina cyanea* (L.)	–	–	–	–	–	10	2/10 (20)
Red-breasted grosbeak *Pheucticus ludovicianus* (L.)	–	–	–	–	–	3	2/3 (67)
Chipping sparrow *Spizella passerina* (Bechstein)	–	–	–	–	–	2	0/2 (0)
Eastern bluebird *Sialia sialis* (L.)	–	–	–	–	–	1	1/1 (100)
Brown thrasher *Toxostoma rufum* (L.)	1	–	–	–	–	–	0/1 (0)
White-crowned sparrow *Zonotrichia leucophrys* (Forster)	–	–	–	–	–	1	0/1 (0)
Clay-coloured sparrow *Spizella pallida* (Swainson)	–	–	–	–	–	1	0/1 (0)
Gambel's white-crowned sparrow *Zonotrichia leuophrys gambelii* (Nuttall)	–	–	–	–	–	1	0/1 (0)
Hermit thrush *Catharus guttatus* (Pallas)	–	–	–	1	–	1	1/1 (100)
Total	1	2	1	4	1	89	32/98 (33)

Note: *brun., Ixodes brunneus; dent., Ixodes dentatus*.
L, larva(e); N, nymph(s); F, female(s).

the *Amblyomma* spp. ticks was tested for *B. burgdorferi*. Prevalence of ticks on birds was not calculated because bird banders did not collect ticks when they were short-staffed, especially during peak migration. Birds had to be processed promptly to avoid undue stress during the holding period in cloth bags.

Figure 2. Veery, *Catharus fuscescens*, parasitized on the lower edge of the right eye by a fully engorged *Ixodes scapularis* nymph. This feeding site is safe from preening. Photo credit: John Woodcock.

The clay-coloured sparrow *Spizella pallida* (Swainson) and the Gambrel's white-crowned sparrow *Zonotrichia leucophrys gambelii* (Nuttall) are first-time host records of *I. scapularis*.

Discussion

This study provides novel findings of ixodid ticks on passerines in central and eastern Canada, and validates the wide dispersal of ticks by migratory songbirds. Our findings of bird parasitism and *B. burgdorferi* infections reinforce the importance of south-to-north, long-distance dispersal of ticks during spring migration, especially from Lyme disease endemic areas to new foci across Canada. Our data indicate that songbird-transported ticks, in particular *I. scapularis*, can contribute to the incidental occurrence of Lyme disease in people and domestic animals in Canada east of the Rockies.

Incidence of **B. burgdorferi** *in bird-transported* **I. scapularis**

Widespread dispersal of Lyme disease spirochetes is facilitated by bird-transported ticks during spring migration in Canada (Table 1). Our data reveal that 31 (35%) of 89 *I. scapularis* nymphs removed from northward-migrating birds were infected with *B. burgdorferi*. Since peak spring migration coincides with heightened questing activity of *I. scapularis* nymphs, ground-frequenting passerines are likely to become parasitized by these immature ixodids whenever they make landfall to replenish energy reserves. *Ixodes scapularis* nymphs comprised the overwhelming majority of bird-feeding ticks that we recorded during northward spring migration. En route, these nymphal ticks are haphazardly released across southern Canada at subsequent stopovers and, within 6–7 weeks, will moult to adults. Our findings are significantly higher than the 15.4% positivity with *B. burgdorferi* for *I. scapularis* nymphs reported by other Canadian researchers (Ogden et al. 2008). However, in their study, there may have been higher degradation of *B. burgdorferi* DNA between the field collection and the laboratory molecular analysis because the ticks were put in sterile tubes and, with no source of added humidity, *I. scapularis* immatures normally die much quicker and spirochetal DNA is lost. We surmise that the higher prevalence of *B. burgdorferi* infection in our study, which was conducted 4–7 years after the Ogden et al. (2008) study, was likely due to higher relative humidity for the live ticks and, additionally, biotechnological advances in DNA extraction and PCR amplification.

New host records for **Ixodes scapularis** *on birds*

We document *I. scapularis* parasitizing two species of birds not previously reported as hosts. An *I. scapularis* nymph was collected from a clay-coloured sparrow *Spizella pallida*, at the southern tip of Thunder Cape on Sibley Peninsula, Ontario, on 19 May 2012. This finding is the first reported record of *I. scapularis* on this bird species. As well, a nymphal *I. scapularis* was collected from a Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii* at the same location on 6 May 2012. With the addition of these two new bird species, a total of 78 wild bird species have now been reported as hosts for *I. scapularis* in North America.

During autumn bird banding, an *I. scapularis* nymph was removed from a northern waterthrush, *Seiurus noveboracensis* (Gmelin), at Thunder Cape, Ontario, on 19 August 2011. This represents the first reported bird parasitism of *I. scapularis* on a passerine during southward autumn migration in northern Ontario. Also, of significance, we document the first live culture of *B. burgdorferi* from an *I. scapularis* nymph collected from a bird, namely a Swainson's thrush, *Catharus ustulatus* (Nuttall), in northern Ontario (Thunder Cape).

New host records for **Ixodes scapularis** *in Canada*

Two partially engorged *I. scapularis* were collected on 7 May 2010 from a worm-eating warbler *Helmitheros vermivorus* (Latham) at Long Point, Ontario, which is endemic for Lyme disease; this host record is the first report in Canada. One of these nymphs was infected with *B. burgdorferi*. This warbler, which overwinters in the West Indies and Central America, was initially captured on 2 May 2010 and recaptured on 3, 4 and 7 May. Based on the extent of engorgement, these ticks had only fed for 3 days. Normally, replete *I. scapularis* nymphs detach within 5 days. Since this neotropical migrant had been in the vicinity of Long Point for at least 5 days, these two ticks must have been acquired locally.

Ixodes scapularis *on a songbird in Prince Edward Island*

We document the first record of *I. scapularis* on a songbird in P.E.I.. Two fully engorged *I. scapularis* nymphs were collected on 21 May 2012 from a common yellowthroat *Geothlypis trichas* (Gmelin), after it was struck by an automobile at Brudenell, P.E.I. These two nymphs moulted to adults (one male, one female). The female moulted in 51 days and, subsequently, tested positive for *B. burgdorferi* using PCR amplification; a live culture was not obtained. Not only is this noteworthy discovery a first for *I. scapularis* on a bird in P.E.I., it provides the premiere account of a songbird-transported tick infected with *B. burgdorferi* in this maritime province. The male *I. scapularis* moulted in 53 days and was negative for *B. burgdorferi.* Biogeographically, the common yellowthroat winters in the southern United States and West Indies and would introduce these fully engorged ticks during trans-border spring migration. Unexpectedly, bird banding programs were discontinued prior to our study, so we were not able to fully monitor the pattern of bird parasitism in the Maritime Provinces.

Amblyomma *species imported to Canada*

We report two species of *Amblyomma* on migratory neotropical passerines in eastern Canada. An engorged *Amblyomma sabanerae* nymph was collected from a veery *Catharus fuscescens* (Stephens), on 25 May 2010 at Thunder Cape, Sibley Peninsula, Ontario; this collection represents the first record of this tick species in northwestern Ontario. For confirmation of tick species identification, this tick underwent DNA sequencing using the 12S*r*DNA gene and was deposited with accession number RML 124515 in the U.S. National Tick Collection, Georgia Southern University, Statesboro, Georgia, United States of America. In addition, an *Amblyomma longirostre* larva was removed from a grey-cheeked thrush *Catharus minimus* (Lafresnaye) on 30 May 2010 at Toronto, Ontario. This tick is the first molecularly confirmed *A. longirostre* larva collected in Canada. The identification of this tick species was confirmed using DNA sequencing, and the tick was retained under accession number RML 124514. Additionally, we collected two unidentified *Amblyomma* (one nymph, one larva). Since *Amblyomma* larvae do not overwinter in Canada, they were directly introduced by migratory passerines from the neotropics during northward, long-distance, cross-border migration.

Ixodes muris *on songbirds*

We document two first-time accounts of the mouse tick *Ixodes muris* Bishopp and Smith on passerines in Quebec and Ontario. An *I. muris* larva was collected from a Lincoln's sparrow *Melospiza lincolnii* (Audubon) on 12 September 2011 at Ste-Anne-de-Bellevue, Quebec; the presence of this larva on a southbound, neotropical songbird indicates that this tick species is established in this province. As well, a *B. burgdorferi*-infected *I. muris* female was removed from a hermit thrush *Catharus guttatus* (Pallas) on 12 August 2012 at Toronto, Ontario; this comprises the first record of a *B. burgdorferi*-positive *I. muris* on a songbird in Ontario. Dolan et al. (2000) found that *I. muris* is capable of acquiring and transmitting Lyme disease spirochetes to mice, but this tick species has reduced vector competence relative to *I. scapularis*. Our findings provide additional evidence that *I. muris* acts as a vector of *B. burgdorferi*, and has the potential to transmit Lyme disease spirochetes to subsequent hosts, such as passerines, small mammals, dogs and cats (Gregson 1956; Scott, J.D., unpublished data).

Borrelia burgdorferi-*infected* **I. scapularis** *on songbirds in Manitoba*

We report new records of *B. burgdorferi*-infected ticks on songbirds in Manitoba. A single engorged *I. scapularis* nymph was removed from a Swainson's thrush on 14 May 2010 at Delta Marsh, Manitoba; this nymph was positive for *B. burgdorferi*. As well, two engorged *I. scapularis* nymphs were collected from a common yellowthroat on 18 May 2010 at the same location. One of these nymphs was positive for *B. burgdorferi*. There are at least two possible scenarios for one nymph being positive and the other not. As larvae, one may have fed on a *B. burgdorferi*-infected host, such as a mouse, while the other fed on a spirochete-free host, such as a chipmunk. Alternatively, one nymph may have fed at a non-infected, cutaneous area on the common yellowthroat, whereas the other fed at a site that was spirochetemic. The initial *B. burgdorferi* infection normally takes several days to fully disseminate throughout the body.

In addition, an engorged *I. scapularis* nymph was collected from a veery *Catharus fuscescens* (Stephens) on 18 May 2010 at Delta Marsh, Manitoba; this tick was also positive for *B. burgdorferi*. Three engorged *I. scapularis* nymphs were removed from a brown-headed cowbird *Molothrus ater* (Boddaert) on 3 June 2010 at Delta Marsh, Manitoba; one of the nymphs produced the first live culture of *B. burgdorferi* from a songbird-transported tick in this province. Of significance, an engorged *I. scapularis* nymph was collected from a mourning warbler *Oporornis formosus* (Wilson) on 3 June 2010 at the same location. This bird parasitism constitutes the first reported *B. burgdorferi*-infected *I. scapularis* on this bird species in this province. Of note, the seroprevalence of *B. burgdorferi* in dogs living in Manitoba was reported by Villeneuve et al. (2011) to be higher than in any other Canadian province.

Borrelia burgdorferi-*positive rabbit tick on a songbird*

We document the first *B. burgdorferi*-infected rabbit tick, *H. leporispalustris*, collected from a songbird in Canada. A live, fully engorged nymph was removed from a

Swainson's thrush on 6 September 2011 at Long Point, Ontario. The Swainson's thrush, a species noted as being a reservoir-competent host (Hamer et al. 2012), may have been spirochetemic for *B. burgdorferi*. Pertinent to Canada, Banerjee et al. (1995) isolated *B. burgdorferi* from a female and detected it in three females and one nymph of *H. leporispalustris* collected from a snowshoe hare, *Lepus americanus* Erxleben, near Grande Prairie, Alberta. In our study, the *B. burgdorferi*-infected *H. leporispalustris* nymph may have acquired the infection by feeding as a larva on a spirochetemic host, and harboured the borrelial infection through the larva-nymph moult. In the upper mid-Western United States, Hamer et al. (2011) also detected *B. burgdorferi* in *H. leporispalustris* parasitizing birds. Pertaining to other tick species on lagomorphs in Canada, Lyme disease spirochetes have been detected in the rabbit-associated tick *Ixodes dentatus* Marx that also feeds on rabbits (Scott et al. 2012) (Table 1); it is noteworthy that this tick species will occasionally parasitize people.

Zoonotic implications of B. burgdorferi-*infected bird-feeding ticks*

Migratory songbirds are dispersal vehicles of ectoparasitic ticks harbouring an extensive array of viral, bacterial and protozoan pathogens that could be transmitted to people and other suitable hosts. In particular, *B. burgdorferi*-infected *I. scapularis* can initiate Lyme disease in humans causing a myriad of symptoms (Cameron et al. 2014; Adrion et al. 2015). Without early or adequate treatment, Lyme disease patients may develop a latent infection because spirochetes will invade and lodge in immune-privileged sites and deep-seated tissues, such as tendons and ligaments (Häupl et al. 1993; Müller 2012), brain (Oksi et al. 1996; MacDonald 2007; Miklossy 2011), bone (Oksi et al. 1994; Fein and Tilton 1997), muscle (Frey et al. 1998), eye (Preac-Mursic et al. 1993), glial and neuronal cells (Ramesh et al. 2008, 2013) and fibroblasts/scar tissue (Klempner et al. 1993). In these secluded niches, *B. burgdorferi* can flourish and, a clinically non-apparent infection, can progress to manifest disease due to the combined effects of a persistent infection and the ensuing inflammatory responses. This can eventuate into a virulent Lyme spirochetosis that can be problematic and difficult to treat. Persistence of *B. burgdorferi* has been well documented in a least 12 different mammalian hosts, including humans (Schmidli et al. 1988; Priem et al. 1998, Li et al. 2011; Hodzic et al. 2014; Middelveen et al. 2014). Pathologically, *B. burgdorferi* can not only circumvent and evade the human immune system but, at the same time, invade host tissue (Kraiczy et al. 2001) and cause inflammation (Ramesh et al. 2013). As Lyme disease advances in human patients, *B. burgdorferi* will: (1) cause immune dysfunction (Stricker and Winger 2001; Hastey et al. 2012), (2) sequester inside cells (Ma et al. 1991; Häupl et al. 1993; Dorward et al. 1997), (3) take on pleomorphic forms, such as blebs and round bodies (Meriläinen *et al.* 2015) and (4) hide in biofilms (Sapi et al. 2011, 2012). Lack of effective treatment for Lyme disease may sometimes result in fatal outcomes (Liegner et al. 1997).

Using non-human primates, namely *Macaca mulatta* (Zimmermann), Embers et al. (2012) demonstrated persistent *B. burgdorferi* infection after 90 days of antibiotic treatment. Often, Lyme disease is refractory to short-term, conventional antimicrobial treatment in advanced cases. Commercial laboratory testing, which is based on immune response, yields poor results for Lyme disease, especially in the advanced stage of this zoonosis, and has a sensitivity of only 46–53% in patients who have surpassed the early disseminated stage (Durovska et al. 2010; Stricker and Johnson 2014).

Long-term antimicrobial treatment is generally needed to kill the diverse pleomorphic forms of *B. burgdorferi* (spirochetes, blebs, or L-form, round bodies, granules and biofilm-like colonies) (Sapi et al. 2011, 2012). During treatment, Lyme disease patients will often have a Jarisch–Herxheimer reaction that manifests with profound fatigue and flu-like symptoms (Bryceson 1976), presumably due to host cytokines released in response to immunogenic debris and neurotoxins from the causal organism (Zajkowska and Hermanowska-Szpakowicz 2002).

Sperling et al. (2012) point out several shortfalls in serological testing and current Lyme disease guidelines in Canada. Unless the populace is aware of the wide distribution of songbird-transported ticks vectoring Lyme disease, unsuspecting residents may be taken off guard when *B. burgdorferi*-infected ticks are dropped in their locality and, subsequently, bite them or their companion animals. In the absence of a tick vector, *B. burgdorferi* may be sexually transmitted to another person (Middelveen et al. 2015).

In summary, our data highlight the fact that at least 78 bird species are avian hosts of *I. scapularis* east of the Rocky Mountains and contribute to the spread of Lyme disease nationwide. With the discovery of *I. scapularis* on a songbird in P.E.I., our findings encapsulate the presence of Lyme disease vector ticks on wild birds across Canada in all provinces and the Yukon. Because songbirds widely disperse *B. burgdorferi*-infected ticks countrywide, health-care professionals, public health officials and the general public must be vigilant of the fact that humans can encounter ticks across Canada capable of transmitting *B. burgdorferi* which can culminate in a pernicious, life-altering infection.

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