Songbird-transported tick *Ixodes minor* (Ixodida: Ixodidae) discovered in Canada

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Abstract—Ticks are carried into Canada by millions of birds during spring migration, and some of these blood-sucking ectoparasites harbour tick-associated pathogens. During a pan-Canadian study of ticks on avian hosts, we identified an extralimital tick, *Ixodes minor* Neumann (Ixodida: Ixodidae) collected from a Common Yellowthroat, *Geothlypis trichas* (Linnaeus) (Passeriformes: Parulidae) at Toronto, Ontario, Canada. Although the *I. minor* larva was not tested for any tick-borne pathogens, this tick species is considered a competent enzootic vector of the Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner *sensu lato* (*s.l.*). Epidemiologically, diverse *B. burgdorferi s.l.* genospecies have been detected in, or isolated from *I. minor*, and this tick species potentially represents a public health risk not only in the southeastern United States of America, where *I. minor* is indigenous, but also in Canada. This tick collection is the northernmost report of *I. minor* in North America, and constitutes the first documentation of this tick species in Canada.

Résumé—Au printemps, durant leur migration vers le Nord, des millions d'oiseaux chanteurs introduisent des tiques au Canada et certaines de ces suceuses de sang, dites ectoparasites, colportent des pathogènes généralement liés à la tique. Durant une étude pancanadienne sur les oiseaux hôtes, on a identifié une tique extra-limite, *Ixodes minor* Neumann (Ixodida: Ixodidae), recueillie sur une Paruline masquée, *Geothlypis trichas* (Linnaeus) (Passeriformes: Parulidae), à Toronto, en Ontario, Canada. Bien que la larve *I. minor* n'ait pas été examinée pour des pathogènes reliés à la tique, cette espèce de tique est censée être un vecteur compétent d'enzootique du spirochète de la maladie de Lyme, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt et Brenner *sensu lato* (*s.l.*). En ce qui concerne les épidémies, différentes espèces génétiques de *B. burgdorferi s.l.* ont été détectées dans ou isolées de *I. minor*, et cette espèce de tique représente un risque pour le public, non seulement dans le Sud des États-Unis, mais aussi au Canada. Ce rapport sur le parasitisme d'oiseaux chanteurs est le plus septentrional qui soit en ce qui concerne *I. minor* en Amérique du Nord et constitue la première documentation sur cette espèce de tique au Canada.

The tick, *Ixodes minor* Neumann (Ixodida: Ixodidae), a blood-sucking ectoparasite of birds and small mammals, is indigenous along the coastal southeastern United States of America (South Carolina, Georgia, Florida) (Clark *et al.* 1998, 2001) and in Central America (Guglielmone *et al.* 2003). This tick species serves as an enzootic vector of the spirochetal bacterium, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner *sensu lato* (*s.l.*), which causes Lyme disease (Lyme borreliosis) (Oliver *et al.* 2003), and manifests in humans as a multisystemic illness. If left untreated or inadequately treated, this spirochetosis can be a persistent infection and, eventually, may result in neurological paralysis, blindness, dementia, total disability and, in some cases, result in fatal outcomes (Liegner *et al.* 1997; Cameron 2010). Worldwide, the *B. burgdorferi s.l.* complex includes at least 21 different genospecies, of which *B. chilensis* Ivanova *et al.* (2013) is the most recently documented and, in the southeastern United States of America, five (*B. americana* Rudenko *et al.*; *B. andersonii* Marconi *et al.*; *B. bissettii* Postic *et al.*; *B. carolinensis* Rudenko *et al.*;

Received 26 October 2013. Accepted 20 January 2014. First published online 5 June 2014.

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Subject editor: Heather Proctor
doi:10.4039/tce.2014.34

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https://doi.org/10.4039/tce.2014.34 Published online by Cambridge University Press

and *B. burgdorferi sensu stricto* (*s.s.*) Baranton *et al.*) have been identified in people, ticks, birds, and rodents (Felz *et al.* 1999; Oliver *et al.* 2003; Rudenko *et al.* 2009a, 2009b; Clark *et al.* 2013). Based on DNA sequencing and phylogenetic analysis, Lin *et al.* (2004) showed significant genetic diversity of *Borrelia* isolates from ticks and rodents collected in the southeastern United States of America. These diverse genotypes can be present in *I. minor*, and dispersed locally or transcontinentally by songbirds (Scott and Durden 2009; Ogden *et al.* 2011). During a borrelial study, Durden *et al*. (1997) isolated *B. burgdorferi s.l.* from the blood of a Northern Cardinal, *Cardinalis cardinalis* (Linnaeus) (Passeriformes: Cardinalidae), parasitised by *I. minor* and from 23 of 56 (43%) immature *I. minor* collected from birds in the coastal southeastern United States of America.

Immatures (larvae, nymphs) of *I. minor* parasitise several ground-frequenting songbirds (Passeriformes), including the House Wren, *Troglodytes aedon* Viellot (Passeriformes: Troglodytidae), Eastern Towhee, *Pipilio erythrophthalmus* (Linnaeus) (Passeriformes: Emberizidae) (Keirans and Clifford 1978) and, likewise, the Carolina Wren, *Thryothorus ludovicianus* (Latham) (Passeriformes: Parulidae), Northern Cardinal, and Northern Waterthrush, *Seiurus noveboracensis* (Gmelin) (Passeriformes: Parulidae) (Durden *et al.* 2001). Both southern temperate and Neotropical passerines annually transport *B. burgdorferi*-infected ticks from the southeastern United States of America to distant northern breeding grounds during spring migration (Scott *et al.* 2001; Morshed *et al.* 2005; Ogden *et al.* 2008; Scott and Durden 2009; Scott *et al.* 2010; Hamer *et al.* 2012; Scott *et al.* 2012). Migratory songbirds have the flight speed and capacity to fly long distances in short periods of time, which potentially allows them to carry engorged ticks from the southern United States of America to Canada before these ixodid ectoparasites complete their blood meals. Therefore, they can introduce a diversity of *B. burgdorferi s.l.* genotypes associated with Lyme disease and other tick-associated pathogens (Scott and Durden 2009; Crowder *et al.* 2010; Mathers *et al.* 2011). Since *I. minor* ticks are considered to have vector competence for *B. burgdorferi s.l.*, they can retain spirochetes in their midgut, and infect suitable zoonotic hosts, such as rodents and songbirds in their new locales.

With respect to mammalian hosts in the southeastern United States of America, *I. minor* has been collected from the cotton mouse, *Peromyscus gossypinus* (Le Conte) (Rodentia: Cricetidae); hispid cotton rat, *Sigmodon hispidus* Say and Ord (Rodentia: Cricetidae); eastern woodrat, *Neotoma floridana* (Ord) (Rodentia: Cricetidae); house mouse, *Mus musculus* Linnaeus (Rodentia: Muridae); and Norway rat, *Rattus norvegicus* (Berkenhout) (Rodentia: Muridae) (Oliver *et al.* 1995, 2003), and these rodent hosts can maintain *B. burgdorferi s.l.* infections in their bodies for years (Oliver *et al.* 2003). The latter two rodents are common in parts of Canada, and could act as vertebrate hosts for *I. minor*.

The present study elucidates long-distance dispersal patterns of extralimital ticks in Canada. Stutchbury *et al.* (2009) used light-level geolocators on certain Neotropical songbirds, and documented the pace of long-distance flight for these passerine migrants to be more than 575 km/day. In our study, the flight path from Savannah, Georgia, United States of America to Toronto, Ontario, Canada is ~1300 km, and can be flown in three to five days by Neotropical and southern-temperate migrants. This is the normal period of time for a larval or nymphal *I. minor* to become fully engorged, and have a complete blood meal. Upon arrival, a replete *I. minor* larva may drop into cool, moist leaf litter, and moult to a nymph and, subsequently, the unfed nymph can parasitise a songbird or small mammal. Of note, Savannah, Georgia is mid-range for indigenous *I. minor* (Durden and Keirans 1996), and the locality of the first North American collection of *I. minor*; at that time, Smith and Gouck (1947) described it as *Ixodes bishoppi* Smith and Gouck, but this name was later shown to be a junior synonym of *I. minor*.

At least six *Ixodes* species ticks are known to be carried into central and eastern Canada during spring migration. The blacklegged tick, *Ixodes scapularis* Say, is a competent vector of *B. burgdorferi s.l.*, and is the most common *Ixodes* tick imported by migratory songbirds. As well, five other *Ixodes* species; namely, *Ixodes affinis* Neumann; *Ixodes brunneus* Koch; the rabbit-associated tick, *Ixodes dentatus* Marx; the mouse tick, *Ixodes muris* Bishopp and Smith, and newly recorded species, *I. minor*, are transported into Canada. Of these six bird-feeding *Ixodes*

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ticks, all are reported in the southeastern United States of America (Durden and Keirans 1996).

Pertinent to our study, *I. minor* is known to harbour at least four *B. burgdorferi s.l.* genospecies: *B. americana*, *B. bissettii*, *B. carolinensis*, and *B. burgdorferi s.s.* (Oliver *et al.* 1996, 2003; Rudenko *et al.* 2009a, 2009b). Recently, Rudenko *et al.* (2014) elucidated cross-species recombination of genes normally found in *B. burgdorferi s.s.* in another *Borrelia* genospecies (*B. americana*) isolated from *I. minor* immatures collected from a Carolina Wren in South Carolina. Hence, *I. minor* can act as a vector of bird-associated strains of Lyme disease spirochetes, and exacerbate a public health risk. After bird-conveyed *I. minor* detach in northern latitudes and, subsequently moult, *B. burgdorferi s.l.* variants may be introduced into the mammalian population, especially rodents. Although vector competence studies have not been done in the laboratory, *I. minor* shows a positive association with *B. burgdorferi s.l.* infection in rodents and songbirds (Clark *et al.* 2001).

During the physical examination of passerines, a partially engorged larval tick was collected from a Common Yellowthroat, *Geothlypis trichas* (Linnaeus) (Passeriformes: Troglodytidae), at Toronto, Ontario, Canada, on 20 May 2013. Using the ixodid larval key of Clifford *et al.* (1961), we initially identified the specimen as *I. minor*. The physical characteristics of our specimen were compared with larval progeny of a bred *I. minor* male and female and, similarly, with scanning electron micrographs of a confirmed (bred) larval *I. minor* that will be used in a forthcoming guide to larval ixodids of the United States of America, which also includes Canadian species, by L.A.D. and colleagues (in preparation). The bred specimens are in the United States National Tick Collection (Georgia Southern University, Statesboro, Georgia, United States of America). This novel, bird-tick finding is the northernmost discovery of this tick species, and constitutes the premiere recognition of *I. minor* in Canada. Because of the special significance of this extralimital tick, we have not tested it for *B. burgdorferi*, and have kept it intact as a voucher specimen; it has been placed in the Biodiversity Institute of Ontario (University of Guelph, Guelph, Ontario, Canada) with accession number, BIO-13–116.

Borrelia burgdorferi s.l. elicits variable antibody responses in infected Lyme disease patients (Stricker 2007). Conventional Lyme disease serology testing often misses the presence of antibodies to *B. burgdorferi s.l.*, especially in immune-suppressed patients (Cadavid *et al.* 2000). Because Lyme disease serology test kits only demonstrate antibodies against 1–3 strains, they can miss antibodies to several *Borrelia* group variants, including those noted in the southeastern United States of America. Some researchers report true sensitivity of an estimated 44–56% for the two-tier serology system for Lyme disease, and lower, when commercial testing is used in clinical practice evaluation (Stricker and Johnson 2008; Sperling and Sperling 2009; Sperling *et al.* 2012). However, *I. minor* can introduce genospecies and/or genotypes of *B. burgdorferi s.l.* (*i.e.*, *B. americana*, *B. andersonii, B. bissettii*, *B. carolinensis*, and *B. burgdorferi s.s.*), which are not represented in current Lyme disease serology test kits. With the exception of *B. carolinensis*, all of these genospecies have been detected in humans. Pathologically, Clark *et al.* (2013) recently documented *B. americana*, *B. andersonii*, and *B. burgdorferi s.s.* in Lyme disease patients in Georgia and Florida. Consequently, patients can exhibit false negative serology and, yet, have bona fide Lyme disease (Rudenko *et al.* 2009b, Clark *et al.* 2013). Since migratory passerines introduce an estimated 50 million Lyme disease vector ticks into Canada annually during northward spring migration (Ogden *et al.* 2008; Scott *et al.* 2010), there is ample opportunity for *I. minor* to bring several diverse *B. burgdorferi s.l.* variants from the southern United States of America, including recombinant genotypes.

Although *I. minor* seldom bites humans (Oliver *et al.* 2003), this tick may introduce new *B. burgdorferi s.l.* genotypes into reservoir-competent, mammalian hosts in new foci. Regardless of where Canadian patients reside, they may become infected with pathogenic Lyme disease-causing spirochetes harboured by songbird-transported *Ixodes* ticks from the southeastern United States of America. Notably, *I. minor* extends its extralimital range into Canada, and is one of six *Ixodes* tick species that can introduce genetically diverse variants of *B. burgdorferi s.l.*

Acknowledgements

The authors thank Bronwyn Dalziel and Nigel Shaw for making the tick collection. They are

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https://doi.org/10.4039/tce.2014.34 Published online by Cambridge University Press

indebted to Irene Pages for French translation of the Abstract. For financial support, they are grateful to the Canadian Lyme Disease Foundation and Lyme Ontario.

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