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First report of a blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodidae), parasitizing a raptor in Canada

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Abstract

We document the first report of a blacklegged tick, *Ixodes scapularis* Say, parasitizing an American Kestrel, *Falco sparverius* Linnaeus (Falconiformes: Falconidae), in Canada. A fully engorged *I. scapularis* nymph was collected from the base of the tongue of an American Kestrel nestling recovered at Mirabel, Québec. This nestling had recently fledged the nest, and was exposed to *I. scapularis* immatures that were host-seeking in the surrounding low-level vegetation. DNA barcoding was used to confirm the identification of the tick. Primers of the flagellin (*fla*) gene were employed to determine whether the Lyme disease spirochete, *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt & Brenner, was present in the *I. scapularis* nymph; the tick was negative. We provide the first report of *I. scapularis* parasitizing a raptor in Canada and, likewise, the first account of this tick species attached to the oral cavity of a bird. Moreover, this bird parasitism is the first documentation of a tick on a falconid bird in Canada.

Key words: American Kestrel; *Falco sparverius*; blacklegged tick; *Ixodes scapularis*; raptor; bird parasitism; DNA barcoding; Canada

Introduction

The American Kestrel, *Falco sparverius* Linnaeus (Falconiformes: Falconidae), is indigenous in different parts of the Western Hemisphere. In Canada, the summer breeding range extends from New Brunswick to British Columbia and the Yukon. Across much of Canada, this bird of prey is migratory; however, in the southern part of the country some birds remain year-round. Unlike other raptor species, American Kestrels rear their young in abandoned woodpecker nests, tree holes, and supplementary nest boxes.

American Kestrels consume various small mammals (i.e., mice, voles) and small passerine birds. They also eat insects, especially grasshoppers. These aerial hunters usually seize prey in their talons after swooping down from an elevated perch. American Kestrels have also been observed nabbing flying insects and small birds in mid-air. From the time the clutch of eggs hatch, the nestlings develop in the nest before leaving in approximately 28 d. Since the nestling in our study was estimated to be 3-4 wk of age, it is believed it had fledged the nest early. Nestling are prone to premature fledging, especially if handled by ornithologists after 3 wk (Lesko & Smallwood 2012).

Whenever an American Kestrel dives to the ground to capture a terrestrial mammal, it may encounter ticks that are questing in low-level vegetation and the leaf litter. If it encounters a recently hatched tick egg mass, it can become heavily infested with hundreds of larvae. Because some

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American Kestrels are migratory, these small falcons have the potential to transport tick long distances comparable to migratory songbirds (Scott *et al.* 2001, Morshed *et al.* 2005, Ogden *et al.* 2008, Scott *et al.* 2010, Scott *et al.* 2012, Scott 2015, Scott & Durden 2015a, b, c, d).

In Canada, *Ixodes* species have previously been reported on three bird orders, namely Accipitriformes (diurnal birds of prey), Galliformes (chicken-like birds), and Passeriformes (perching birds). However, there is little known about ticks parasitizing raptors in Canada. The first report of ticks parasitizing a raptor was that of larvae of the avian coastal tick, *Ixodes auritulus* Neumann, attached to a Cooper's Hawk, *Accipter cooperii* (Bonaparte), in British Columbia (Scott *et al.* 2013). Notably, some of the larvae were infected with the spirochetal bacterium *Borrelia burgdorferi* sensu lato (s.l.) Johnson, Schmid, Hyde, Steigerwalt & Brenner, the causative agent of Lyme disease (Burgdorfer *et al.* 1982). Because these larvae had not taken a previous blood meal, it is likely that the Cooper's Hawk was acting as a reservoir for Lyme disease spirochetes.

The blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodida: Ixodidae) (northern populations formerly called *I. dammini*), is an obligate, hematophagous ectoparasite indigenous to North America east of the Rocky Mountains. *Ixodes scapularis* has been documented as far north and as far west as Slave Lake, Alberta (Scott *et al.* 2001; Morshed *et al.* 2005). Blacklegged ticks carry and transmit *Borrelia burgdorferi* s.l. As well, *I. scapularis* can harbor and transmit at least 8 other tick-borne pathogens, namely *Babesia* spp. (e.g., *B. duncani*, *B. microti*), *Bartonella* spp. (e.g., *B. henselae*), *Ehrlichia* spp. (e.g., *E. ewingii*), *Mycoplasma* spp. (e.g., *M. fermentans*), *Anaplasma* spp. (e.g., *A. phagocytophilum*), *Borrelia miyamotoi* (relapsing fever group spirochete), *Ehrlichia muris*-like agent, and Deer Tick Virus (Powassan virus group). Of epidemiological importance, *B. miyamotoi* is pathogenic to humans (Platonov *et al.* 2011, Boden *et al.* 2016), and *I. scapularis* females can pass this tick-borne pathogen via transovarial transmission to eggs and, subsequently, to larvae (Rollend *et al.* 2013); all host-feeding stages (larvae, nymphs, adults) can be infected.

The DNA barcoding method was used to confirm the identification of the *I. scapularis* tick. Although ticks are usually identified using morphological characters, which correspond to published species descriptions, this method can lead to uncertain species determinations. Taxonomic problems can arise when body parts are missing or damaged. Often the hypostome is broken off during tick removal, and is left behind in host tissue. Molecular approaches to identification can resolve these issues by comparing the target specimen to the reference sequence of a positively identified specimen (Chao *et al.* 2011, Fiorini *et al.* 2014).

The aim of this study was to determine whether ticks parasitize raptors in eastern Canada, and ascertain if these ticks are infected with *B. burgdorferi* s.l.

Materials and methods

Tick collection

Using fine-pointed, stainless steel forceps, an ixodid tick was removed from the mouth of an American Kestrel nestling by staff at the Lechoir Wild Bird Conservation Centre at Hudson, Québec on 29 June 2016. Two engorged ticks at the back of the throat were noted, but were not removed. The fledged nestling was found on the floor of an automobile garage at Mirabel, Quebec (45° 39′ N, 74° 05′ W). This nestling was able to walk, but could not fly. There were no visible injuries and no evidence of predation by a cat. The tick was put directly in a 2 ml micro tube containing 94% ethyl alcohol. The tick was sent to the laboratory (JDS) for identification using a taxonomic key (Durden & Keirans 1996).

The anterior section of the dorsal surface to the nymph was photographed using a Nikon Multizoom AZ100M stereoscopic microscope with a Nikon DS-Fi1 camera. The composite, close-

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up photograph consisted of 17 layers. Because of the scientific significance of this engorged nymph, we cut the tick transversely, and tested the posterior part of the idiosoma for *B. burgdorferi* s.l., and retained the anterior section for DNA barcoding and as a voucher specimen.



FIGURE 1. American Kestrel, nestling, 3–4 wk of age, parasitized by an *Ixodes scapularis* nymph at the base of the tongue. Photo credit: Susan Wylie.

DNA extraction and PCR amplification

The posterior section of the tick (idiosoma) was put in a 2 ml micro tube of 94% ethyl alcohol, and sent by courier to the PCR amplification laboratory (JEF). DNA was extracted from the tick using the ammonium hydroxide technique as previously described (Foley & Piovia-Scott 2014). Real-time PCR was used to amplify *B. burgdorferi* s.l. DNA as described earlier (Scott & Foley 2016).

DNA barcoding

DNA barcoding was employed to confirm the tick identification. Total genomic DNA was extracted from the anterior section of the ixodid nymph (barcode number, TJSD014-16) using standard protocols from the Canadian Centre for DNA Barcoding (CCDB, Ivanova *et al.* 2006) with modifications to the centrifugation parameters to accommodate use on a single column (Epoch Biolabs). With the exception of the second wash and the elution spins, the column was balanced each time, and spun at 6000g for 2 min. In the second wash, the column was spun at 10,000g for 4 min with the excess flow-though being disposed before conducting a second spin at 10,000g for an additional 4 min. The final elution step involved spinning the column at 10,000g for 5 min. The DNA barcode region (Hebert *et al*. 2003) of cytochrome *c* oxidase subunit I (COI) was also amplified using standard CCDB protocols (Ivanova & Grainger 2006) consisting of 10.25 µL of both forward and reverse primers, and 2 µL of DNA template. The PCR primers consisted of equal part LepF1/LepR1 (Hebert *et al.* 2004) and LCO1490/HCO212198 (Folmer *et al.* 1994). The thermocycling regime was as follows: 94°C for 1 min, 5 cycles at 94°C for 40 s, 45°C for 40 s, 51°C for 40 s, 72°C for 1 min, with a final extension at 72°C for 5 min. Amplicons were diluted with 30 µL of Hyclone ultra-pure water (Thermo Scientific) and bi-directionally sequenced at the CCDB using a modified BigDye 3.1 Terminator (Applied Biosystems) protocol (Hajibabaei *et al.* 2005) on an ABI 3730XL capillary sequencer (Applied Biosystems). The cycle sequencing regime was: 96°C for 1 min followed by 35 cycles at 96°C for 10 s, 55°C for 5 s, 60°C for 2.5 min, with a final extension at 60°C for 5 min. Trace

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files were assembled, edited and aligned to the barcode region in CodonCode v.4.2.7 (CodonCode Corporation).

The sequence, trace files, and corresponding specimen collection details are available in the Barcode of Life Datasystems (BOLD) dataset DS-ISAK, and can be retrieved by accessing DOI:dx.doi.org/10.5883/DS-ISAK. The sequence was also deposited in GenBank (accession number: KY322738). The specimen voucher (anterior section of the tick) was retained after DNA extraction, and is archived in 95% ethyl alcohol in the Centre for Biodiversity Genomics (CBG) with accession number BIO-16-099. The DNA extract is held at -80°C in the same location.



FIGURE 2. *Ixodes scapularis* nymph (16-5A70); dorsal view showing capitulum and scutum (TJSD014-16). Bar, 0.5 mm. Photo credit: Kellyn Hough.

Results

Using the taxonomic key (Durden & Keirans 1996), the preliminary identification of the fully engorged nymph was *I. scapularis*. The specimen TJSD014-16 (anterior section of tick 16-5A70) was successfully sequenced for the DNA barcode region of COI, with a sequence length of 658 bp (0% ambiguities) with no evidence of stop codons or contamination (Figure 3). Comparison with the DNA barcode library using the BOLD ID Engine resulted in a 100% pairwise nucleotide match to 47 other *I. scapularis* specimens housed in BOLD, further confirming its identification.



FIGURE 3. Illustrative barcode of the *Ixodes scapularis* specimen TJSD014-16 generated in the spider package (Brown *et al*. 2012) in R (R Core Team 2014).

Discussion

This study documents the first record of an *I. scapularis* tick parasitizing an American Kestrel in Canada. Although it is highly unusual, we provide the first account of an ixodid tick parasitizing its host inside the mouth. Since American Kestrels capture and consume small mammals and passerine birds in Lyme disease endemic areas, this small falcon could play a role in the epidemiological transmission cycle of *B. burgdorferi* s.l. and other tick-associated pathogens.

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Established population of I. scapularis

When wild birds are heavily infested with *I. scapularis* larvae and nymphs, they can initiate an established population of blacklegged ticks (Scott *et al*. 2014). The parasitism of an American Kestrel nestling by an *I. scapularis* nymph species in late June signifies the presence of an established population of this tick species at the recovery site. The nestling was parasitized 3 wk after spring migration for passerines, and it would be highly unlikely that a songbird-transported larva would have molted and be questing as a nymph. Larvae take 5 to 7 wk to molt before they become nymphs. Moreover, when nymphs molt to adults in 5 to 9 wk (Scott et al. 2016), they do not parasitize birds. At this northern latitude, the peak questing activity for *I. scapularis* nymphs occurs throughout the month of June (Carey *et al.* 1980, Mannelli *et al.* 1994). Since the American Kestrel nestling could not fly, the tick must have been acquired locally in the area. The timing of this parasitism indicates that *I. scapularis* is established in the Mirabel area.

In a Lyme disease endemic area, small mammals and certain birds act as reservoirs of *B. burgdorferi* s.l. and other tick-associated pathogens (Nicholson *et al.* 2009, Hamer *et al.* 2012). When American Kestrels eat these *B. burgdorferi* s.l.-infected animals and birds, they, too, can become infected. For example, when mallards, *Anas platyrhynchos* Linnaeus were orally inoculated with *B. burgdorferi* s.l., they acquired the infection themselves (Burgess 1989). Additionally, Richter *et al.* (2000) discovered that American Robins, *Turdus migratorius* Linnaeus, were reservoir- competent hosts. Other researchers have cultured *B. burgdorferi* s.l. from skin biopsies (Durden *et al.* 2001), blood and organs of passerines (Anderson & Magnarelli 1984, Anderson *et al.* 1990, McLean *et al.* 1993). In addition, Hamer *et al.* (2012) reported Swainson's Thrush, *Catharus ustulatus* (Nuttall), is a reservoir-competent host.

American Kestrels host I. scapularis

Our findings show that American Kestrels are hosts of *I. scapularis* immatures. Previously, Lesko & Smallwood (2012) reported an *I. scapularis* tick on an American Kestrel nestling in New Jersey, U.S.A., but the vector-host details (i.e., life stage, engorgement, date collected, attachment site, tick identifier) are lacking. Moreover, there is no voucher specimen and no photograph or molecular identification to substantiate this report. Furthermore, there is no explanation of how the tick reached the nest to parasitize a non-fledged nestling. Normally, *I. scapularis* larvae and nymphs quest for hosts in the leaf litter. In the present study, we conducted a taxonomic assessment on the nymphal tick and, to confirm the identification, we used DNA barcoding. In addition, we used molecular analysis to assess whether the *I. scapularis* nymph might be infected with *B. burgdorferi* s.l.

DNA barcoding ticks

DNA barcoding uses a standard 648-bp fragment of the cytochrome *c* oxidase submit I (COI) gene to identify specimens based on a robust library of reference sequences from expertly identified specimens held in the Barcode of Life Data Systems (BOLD, Ratnasingham & Hebert 2007), an online DNA barcode repository. DNA barcoding has been used successfully for specimen identification in many animal groups (Hebert *et al*. 2003, Blagoev *et al.* 2016), including ticks (Erster *et al.* 2013, Lv *et al.* 2014, Lah *et al.* 2016).

For epidemiologists to track the movement of ticks and tick-borne diseases, it is vitally important that ticks be properly identified. In particular, migratory birds can transport ticks thousands of kilometers during transhemispheric and intercontinental migration (Hoogstraal & Kaiser 1961, Olsén *et al.* 1995, Scott & Durden 2015a, Scott & Durden 2015b). Epidemiologically, some of these ticks are infected with tick-borne pathogens. Ultimately, failure to identify ticks correctly can lead to host animals and people not being correctly diagnosed and treated by veterinarians and health-care providers. Barcoding provides a reliable molecular alternative for accurate tick identification.

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Unusual tick attachment site

Attachment of ixodid ticks on the inside of the mouth is an unusual phenomenon. Most ectoparasites, especially ticks, attach to the outer skin when they take a blood meal. In the case of birds, ticks typically attach to the head where the bird is unable to preen. However, presumably when the American Kestrel nestling tried to ingest ticks for food, the ticks attached to the lining of the mouth. One nymphal tick attached to the base of the tongue while the other ticks attached to the back of the mouth. Based on previous bird-tick studies, wild birds eat ticks (Milne 1950, Hoogstraal & Kaiser 1961). In the present study, the ticks may have averted swallowing, and attached to the inside of the mouth. Since we did not conduct fecal studies, we do not know if the American Kestrel nestling actually ingested the replete ticks attached at the back of the mouth. They may have been purged after engorgement, similar to a foreign object being spit out. This unusual bird-tick association not only shows that birds eat ticks, or try to eat ticks, it affirms that *I. scapularis* immatures parasitize raptors. Moreover, the attachment of an ixodid tick in the oral cavity of a bird constitutes the first documentation of this type of parasitism.

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