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Original article

# *Babesia odocoilei* and zoonotic pathogens identified from *Ixodes scapularis* ticks in southern Ontario, Canada

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ABSTRACT

Cervid babesiosis, caused by the protozoan hemoparasite *Babesia odocoilei* and transmitted by the blacklegged tick *Ixodes scapularis*, is an emerging disease of Canadian cervids. This pathogen has not yet been described in humans. Data are lacking on the role of migratory birds in the adventitious spread of *Ba. odocoilei*-infected ticks, as well as on the infection status of *I. scapularis* in environments used by susceptible wildlife hosts. Following a high-mortality outbreak of cervid babesiosis at the Toronto Zoo [TZ], the present study was initiated to investigate *Ba. odocoilei* and other tick-borne pathogens of veterinary and public health importance (*Borrelia burgdorferi* sensu stricto (s.s.), *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, and *Babesia microti*) in *I. scapularis* at three sites in southern Ontario, Canada. Blanket dragging for questing ticks yielded *I. scapularis* from the three sites evaluated: TZ, Point Pelee National Park, and Long Point Bird Observatory [LPBO]. *Babesia odocoilei* was identified in *I. scapularis* collected by dragging at the TZ and at LPBO. *Borrelia burgdorferi* s.s. was identified in *I. scapularis* at all three sites. *Anaplasma phagocytophilum* was identified in *I. scapularis* collected from the TZ. During the springs of 2016 and 2017, 1102 northward-migrating birds were examined for ticks at LPBO. One or more *I. scapularis* were found on 3.2% of birds ($n$ = 595) in 2016, and 6.7% ($n$ = 507) of birds in 2017. Overall, across both years, 0.2% and 0.5% of birds carried one or more *I. scapularis* ticks that tested PCR-positive for *Ba. odocoilei* and *Bo. burgdorferi* s.s., respectively. These data indicate that *Ba. odocoilei*-positive *I. scapularis* are found in southern Ontario, and suggest that bird-borne ticks have the potential to contribute to range expansion of both *Ba. odocoilei* and *Bo. burgdorferi* s.s. in Canada.

## 1. Introduction

The blacklegged tick (*Ixodes scapularis*) is the vector for many pathogens of medical and veterinary importance in North America. The range of this tick is expanding as a result of anthropogenic influences on the environment and climate warming (Leighton et al., 2012). Ticks carrying the causative agents of medically important tick-borne zoonoses, such as Lyme borreliosis (*Borrelia burgdorferi* sensu stricto [s.s.]) and human granulocytic anaplasmosis (*Anaplasma phagocytophilum*), may be transported over vast distances and introduced to new areas by birds in flight (Ogden et al., 2008). The potential role for migratory birds in the northward range expansion of non-zoonotic tick-borne diseases of veterinary importance has received little research attention. One such disease is cervid babesiosis caused by the protozoan hemoparasite *Babesia odocoilei*, for which *I. scapularis* is the definitive host and only known competent vector (Waldrup et al., 1990). *Babesia odocoilei* is endemic in the southeastern United States (Holman et al., 2000) where the natural reservoirs are wild white-tailed deer (*Odocoileus virginianus*). Disease associated with *Ba. odocoilei* (cervid babesiosis) has emerged in captive cervids within Canada since 2012 (Mathieu et al., 2018). In addition, the zoonotic pathogens *Babesia microti*, *Bo. burgdorferi* s.s., *Borrelia miyamotoi*, and *Anaplasma phagocytophilum* share the same tick vector, *I. scapularis*, and are also emerging in Ontario (Dibernardo et al., 2014).

The Toronto Zoo [TZ] in southern Ontario, Canada, is situated within a natural area of forest and wetlands (the Rouge National Urban Park) with abundant free-ranging wildlife including white-tailed deer that are the main blood meal hosts for adult *I. scapularis* (Rand et al., 2003). Such a setting presents a potential risk to the health of zoo animals and humans (staff and visitors) due to the presence of disease-

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carrying vectors. Tick parasitism of zoo animals and staff is occasionally reported, but the likelihood of tick-borne disease transmission has not been investigated. Following a high-mortality outbreak of cervid babesiosis in Eurasian tundra reindeer (*Rangifer tarandus tarandus*) and wapiti (*Cervus canadensis*) at the TZ from 2012 to 2015 (Mathieu et al., 2018), we investigated *Ba. odocoilei* and other tick-borne pathogens in *I. scapularis* at the TZ and two other sites in southern Ontario where *I. scapularis* is endemic (Barker and Lindsay, 2000). Further, we captured northward-migrating birds in southern Ontario in order to assess their tick burdens and thereby to investigate the potential for bird-borne ticks and tick-borne pathogens to contribute to the emergence of *Ba. odocoilei* in regions sensitive to climate change.

Our objectives were: 1) to survey northward-migrating birds captured at Long Point Bird Observatory ([LPBO], Port Rowan, Ontario) for ticks; 2) to survey ticks by blanket dragging from three sites used by wild cervids (TZ, LPBO, and Point Pelee National Park [PPNP]); and 3) to determine the presence or absence of *Ba. odocoilei, Ba. microti, Bo. burgdorferi* s.s., *Bo. miyamotoi*, and *A. phagocytophilum* in host-feeding (bird-borne) and questing *I. scapularis* obtained from these locations by polymerase chain reaction (PCR).

## 2. Materials and methods

### 2.1. Bird sampling and study area

Bird sampling took place during spring migration in May 2016 and May 2017 at LPBO migration monitoring field station in southern Ontario. Mist nets and ground traps were used to capture birds daily from sunrise until noon, in accordance with protocols described by the North American Bird Banding Manual (Gustafson et al., 1997). Each captured bird was identified to species and leg banded with a unique bird identification number. Within-year recaptures were excluded from analysis because ticks found on recaptured birds would have been taken up from existing tick populations at the study site. All birds were checked for ticks; the additional time spent handling birds for this purpose was limited to 3 min per bird. Feathers along apterylae of the head, neck, and body were gently parted and the skin was inspected for ticks using binocular head loupes. All ticks were removed using fine forceps and placed in sterile labelled tubes containing 70% ethanol. Fieldwork was carried out with approvals from the Animal Care Committees of the University of Guelph and the TZ in compliance with the regulations of the Canadian Council on Animal Care.

### 2.2. Tick dragging and opportunistic collection of questing and host-feeding ticks

Tick collection was carried out at the TZ based on the history of clinical *Ba. odocoilei* infection in zoo cervids, and at LPBO and PPNP because these sites host populations of wild white-tailed deer and are known endemic sites for *I. scapularis* (Barker and Lindsay, 2000).

Blanket dragging was used to sample questing ticks at the TZ in October 2016, and July, August, and October 2017; at PPNP in November 2016; and at LPBO in May 2016 and May 2017. Blanket dragging was conducted only on days when surface vegetation was dry to the touch. A 1 $m^2$ white flannel drag cloth attached to a wooden pole was pulled across the surface of ground vegetation in parallel transects for at least 1.5 (and up to 6) person-hours per site. Field personnel checked each drag cloth and their clothing at 3 min intervals, during which time the timer was stopped. At each site, at least 1000 $m^2$ of vegetation were sampled per visit. Staff at the TZ were asked to submit ticks found on their clothing or on animals from May 2016 to November 2017. In addition, bird banders and volunteers at LPBO submitted ticks found on their clothing in May and June 2017. All ticks were placed in sterile labelled tubes containing 70% ethanol immediately after collection.

### 2.3. Taxonomic identification of ticks

Ticks were identified morphologically to species and life stage using a stereomicroscope and taxonomic keys (Keirans and Litwak, 1989) at the University of Guelph (Guelph, Ontario, Canada) and the National Microbiology Laboratory (NML; Winnipeg, Manitoba, Canada).

### 2.4. Polymerase chain reaction (PCR) and DNA sequencing

All ticks identified as *I. scapularis* were tested for the presence of *Ba. odocoilei, Ba. microti, Bo. burgdorferi* s.s., *Bo. miyamotoi* and *A. phagocytophilum*. Only *I. scapularis* were screened for pathogens due to financial limitations of this study. DNA extraction and conventional PCR testing for infection with *Ba. odocoilei* was performed at the University of Guelph. DNA was extracted from individual ticks, except when multiple *I. scapularis* were present on a given bird or were collected from the same environmental location on the same day. In these instances, all ticks collected from the same bird or from the same environmental sample (i.e., same location and date) were pooled by life stage (i.e., all adults, nymphs, or larvae) for DNA extraction. Minimum infection rate (i.e., assuming one positive tick per pool) was used for the interpretation of results (Hamer et al., 2012).

Ticks were bisected with a sterile scalpel blade and homogenized with sterile lysis beads (0.75 *g* of 2 mm dia. zirconia beads and 0.15 *g* of 0.1 mm dia. zirconia/silica beads, BioSpec Products, Bartlesville, Oklahoma, USA) in a sterile tube containing 0.5 mL of DNAzol® Reagent (Invitrogen Life Technologies, Carlsbad, California, USA) for 30 min at 30 Hz with a TissueLyser II (Qiagen, Toronto, Ontario, Canada). For pools, one half of every tick in the pool was added to the same tube. After homogenization, the mixture was incubated overnight at room temperature on a rotator (Fisher Roto-Rack, Model 96, Fisher Scientific Co., Pittsburgh, Pennsylvania, USA), then centrifuged at 4000 × *g* for 2 min. The supernatant was transferred to a sterile microcentrifuge tube to which 0.25 mL of 100% ethanol was added. The tube was inverted 3–6 times, and the samples were incubated for 2 min at room temperature. The mixture was centrifuged at 4000 × *g* for 2 min to pellet the DNA, and the supernatant was discarded. The DNA pellet was washed twice with 0.5 mL of fresh 75% ethanol by gently inverting the tube 3–6 times. Finally, the DNA pellet was air dried and resuspended in 0.1 mL of 8 mM NaOH by slowly passing the pellet through a pipette tip. Insoluble materials were removed by centrifugation at 12,000 × *g* for 10 min. The DNA quality was determined using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) after which sample aliquots were stored at −20 °C until testing for *Ba. odocoilei* at the University of Guelph by PCR. Additional aliquots were then shipped overnight on ice packs to the NML for *Ba. microti, Bo. burgdorferi* s.s., *Bo. miyamotoi,* and *A. phagocytophilum* PCR.

### 2.5. Molecular analysis of tick DNA extracts for Babesia odocoilei and DNA sequencing

Extracted DNA was used as a template in a hemi-nested PCR assay as described by Milnes et al. (2019). Briefly, DNA was first used to amplify partial 18S rDNA amplicons (1393 bp amplicon from primers Piro_18S_300 F and Piro_18S_1688R) from piroplasms (Ramos et al., 2010; Mathieu et al., 2018); following agarose gel electrophoresis and purification of positive samples, a hemi-nested secondary PCR (1147 bp amplicon from primers Cocci_18S_595 F and Piro_18S_1688R) was used to provide sufficient DNA for direct amplicon sequencing as described previously (Milnes et al., 2019). Direct sequencing of *Babesia* sp. amplicons was performed by the Genomics Facility Advanced Analysis Centre, University of Guelph using primers Piro_18S_1688R and Lank_18S_1278R (Milnes et al., 2019). The resulting chromatograms were assembled into consensus contigs using a bioinformatics program (Geneious® 11.0.5., Biomatters, Auckland, New Zealand). Thereafter,

the partial 18S rDNA sequences (trimmed to remove primer binding sites) were searched against the public sequence databases using the basic local alignment search tool (BLAST) algorithm (Altschul et al., 1990).

### 2.6. Molecular analysis of ticks for zoonotic pathogens

Extracted DNA from all ticks identified as *I. scapularis* was submitted to the NML and tested for the presence of *Ba. microti, Bo. burgdorferi* s.s., *Bo. miyamotoi,* and *A. phagocytophilum* using real-time PCR assays as described previously (Dibernardo et al., 2014; Ogden et al., 2006). A multiplex real-time PCR assay was used to screen for *Bo. burgdorferi* s.s. and *A. phagocytophilum* (Courtney et al., 2004), followed by an *ospA* real-time PCR to confirm *Bo. burgdorferi* s.s. infection in multiplex realtime PCR-positive samples (Ogden et al., 2006). Analysis for *Ba. microti* was conducted using methods described by Bullard et al. (2014).

## 3. Results

### 3.1. Bird-borne ticks

Five species of bird-borne tick were identified: *I. scapularis, Ixodes dentatus, Ixodes marxi, Haemaphysalis leporispalustris*, and *Amblyomma americanum*. With the exception of the Carolina wren (*Thryothorus ludovicianus*), which is a year-round resident at LPBO, all tick-infested birds were migratory species that reside in the United States or further south during winter and migrate northwards into Canada for the breeding season (Sibley, 2014).

Of the 595 birds examined in 2016, ticks were found on 3.7% (22/595; Table 1). In total, 47 ticks were found in 2016; however, one tick from a grey catbird (*Dumetella carolinensis*) was observed deep within the ear canal and could not be safely collected, and thus 46 ticks were submitted for identification. *Ixodes scapularis* ticks were carried by 3.2% (19/595) of birds.

In 2017, 507 birds were examined with an overall tick infestation prevalence of 11.2% (57/507; Table 1). One tick from a Carolina wren was observed in the ear canal but could not be safely collected; thus, 167 ticks from 56 birds were collected for identification. *Ixodes scapularis* ticks were carried by 6.7% (34/507) of birds.

### 3.2. Tick dragging and opportunistic collection of questing and host-feeding ticks

In total, five species of ticks (*I. scapularis, Ixodes cookei, I. marxi, H. leporispalustris*, and *Dermacentor variabilis*) were found at the three field sampling sites (Table 2; Fig. 1). Over both years and all collection methods, the numbers of *I. scapularis* ticks collected from each site was 161 from the TZ and 49 from LPBO. PPNP was only sampled in 2016 when blanket dragging yielded 47 adult ticks, of which 41 were *I. scapularis*. Three engorged adult *I. scapularis* were removed by zookeepers from the carcass of a wild white-tailed deer that was predated by coyotes on the TZ grounds in May 2016.

### 3.3. Babesia odocoilei PCR test results

All bird-borne *I. scapularis* ticks collected from 2016 and 2017 ($n$ = 166; 113 nymphs and 53 larvae) were tested by PCR for *Ba. odocoilei*, either individually or pooled by bird and life stage as described previously. Two samples tested positive for *Ba. odocoilei*: a pool of larvae removed from a blue jay in 2016 and a pool of larvae removed from a Swainson's thrush (*Catharus ustulatus*) in 2017. Only 0.2% (2/1,102) of birds sampled harbored *Ba. odocoilei*-positive ticks (Table 1). In addition, both birds were co-infested with *I. scapularis* nymphs, which tested PCR negative for *Ba. odocoilei*. The minimum infection rate of *Ba. odocoilei* in all bird-borne *I. scapularis* ticks over both years was 1.2% (2/166).

All *I. scapularis* ticks collected opportunistically and by blanket dragging from the TZ, PPNP, and LPBO in 2016 and 2017 were tested individually or pooled by date, location, and life stage (Table 2; Fig. 1). Of all the environmentally-collected ticks tested, four samples were positive for *Ba. odocoilei.* A total of 161 *I. scapularis* ticks were collected from the TZ in 2016 and 2017, of which two larval pools tested positive for *Ba. odocoilei* by PCR, giving a minimum infection rate for *Ba. odocoilei* of 1.2% (2/161). At LPBO, one adult and one pool of adult ticks were positive for *Ba. odocoilei*, with a minimum infection rate of 4.1% (2/49). All adult *I. scapularis* from PPNP tested negative for *Ba. odocoilei*.

### 3.4. Zoonotic pathogen PCR test results

All bird-borne *I. scapularis* ticks collected in 2016 and 2017 were tested by PCR for selected zoonotic bacterial and protozoal agents. Overall, 0.5% (6/1,102) of birds carried at least one tick that was positive by PCR for *Bo. burgdorferi* s.s. (Table 1). All *Bo. burgdorferi* s.s. positive ticks were individual or pooled nymphs. The minimum infection rate of *Bo. burgdorferi* s.s. in all bird-borne *I. scapularis* ticks over both years was 3.6% (6/166). No bird-borne ticks tested positive for *A. phagocytophilum, Bo. miyamotoi,* or *Ba. microti*.

All *I. scapularis* ticks collected from birds and by blanket dragging at the TZ, PPNP, and LPBO in 2016 and 2017 were tested for zoonotic pathogens (Table 2; Fig. 1). The minimum infection rate of *Bo. burgdorferi* s.s. was 0.6% (1/161) at the TZ, 4.9% (2/41) at PPNP, and 4.1% (2/49) at LPBO. The engorged *I. scapularis* recovered from a wild deer carcass at the TZ were pooled, and DNA extracted from these ticks tested PCR-positive for *A. phagocytophilum*, giving a minimum infection rate for *A. phagocytophilum* of 0.6% (1/161) across all ticks collected from TZ. None of these ticks tested positive for *Ba. microti* or *Bo. miyamotoi.*

### 3.5. DNA sequencing to confirm Babesia odocoilei in PCR-positive ticks

The six *Ba. odocoilei* PCR amplicons each provided 1071 bp of 18S rDNA sequence data for comparison among samples and with public databases. Four of 6 amplicons (these were: 1. a pooled homogenate of unfed larval ticks collected by blanket dragging at TZ in 2016; 2. a pooled homogenate of unfed larval ticks collected by blanket dragging at TZ in 2017; 3. an individual unfed adult tick collected by blanket dragging at LPBO in 2017; 4. a pooled homogenate of unfed adult ticks collected by blanket dragging at LPBO in 2017) had 100% identity over their entire lengths to each other and to the sequences obtained from clinical cervid babesiosis cases in the TZ reindeer (GenBank accession number MF357057) and wapiti (MF357056) during the 2012–2015 outbreak (Mathieu et al., 2018). These four samples also showed 100% identity with *Ba. odocoilei* isolated from wild white-tailed deer in Texas (U16369.2). A representative sequence from this study (originating from a questing adult *I. scapularis* collected by blanket dragging from LPBO in 2017) was submitted to GenBank with accession number MH366540. The fifth sample, a pooled homogenate of larval ticks removed from a blue jay in 2016 (GenBank accession number MH899097), had a single nucleotide difference over its 1071 bp length from the previous sequences (99.9% pairwise identity). The final sequence, a pooled homogenate of larval ticks removed from a Swainson's thrush in 2017, was more divergent than the previous 5 sequences. This sequence (GenBank accession number MH366631) had 5 nucleotide differences (99.5% sequence identity) to the majority of sequences obtained from *Ba. odocoilei*-positive ticks in the present study and to cervid isolates noted above.

## 4. Discussion

This study was initiated to investigate the eco-epidemiology of *Ba. odocoilei* in southern Ontario, Canada, following an outbreak of *Ba.*

**Table 1**
Bird species that harbored ticks in spring 2016 and 2017 at Long Point Bird Observatory, Ontario, Canada, and the results of pathogen testing[a] of *Ixodes scapularis* ticks.

| Avian species on which ticks were identified | | No. of birds examined | | Total no. of birds carrying ticks | | No. of birds carrying ticks of each species (no. of birds carrying *Ixodes scapularis* ticks PCR-positive for *Babesia odocoilei* / no. of birds carrying *Ixodes scapularis* ticks PCR-positive for *Borrelia burgdorferi* s.s.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Ixodes scapularis* | | *Ixodes dentatus* | *Ixodes marxi* | *Haemaphysalis leporispalustris* | | *Amblyomma americanum* | Unidentified tick species | |
| | | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2017 | 2016 | 2017 |
| American robin | *Turdus migratorius* | 0 | 5 | 0 | 3 | 0 | 1 (0/0) | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Baltimore oriole | *Icterus galbula* | 0 | 12 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Blue jay | *Cyanocitta cristata* | 44 | 58 | 5 | 9 | 5 (1/2) | 7 (0/2) | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Brown-headed cowbird | *Molothrus ater* | 5 | 0 | 2 | 0 | 2 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Canada warbler | *Cardellina canadensis* | 0 | 9 | 0 | 1 | 0 | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Carolina wren[b] | *Thryothorus ludovicianus* | 2 | 1 | 1 | 1 | 1 (0/0) | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| Chipping sparrow | *Spizella passerina* | 2 | 0 | 1 | 0 | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Common grackle | *Quiscalus quiscula* | 3 | 3 | 1 | 1 | 1 (0/0) | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Common yellowthroat | *Geothlypis trichas* | 27 | 27 | 2 | 4 | 2 (0/0) | 3 (0/1) | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| European starling | *Sturnus vulgaris* | 0 | 1 | 0 | 1 | 0 | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Grey catbird | *Dumetella carolinensis* | 38 | 28 | 3 | 4 | 1 (0/0) | 1 (0/0) | 1 | 0 | 0 | 3 | 0 | 1 | 0 |
| Grey-cheeked thrush | *Catharus minimus* | 0 | 11 | 0 | 5 | 0 | 3 (0/0) | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Indigo bunting | *Passerina cyanea* | 0 | 3 | 0 | 3 | 0 | 3 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lincoln's sparrow | *Melospiza lincolnii* | 17 | 8 | 2 | 5 | 2 (0/0) | 1 (0/0) | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Mourning warbler | *Geothlypis philadelphia* | 6 | 10 | 1 | 1 | 1 (0/0) | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Northern waterthrush | *Parkesia noveboracensis* | 8 | 6 | 1 | 1 | 1 (0/0) | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Red-eyed vireo | *Vireo olivaceus* | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Red-winged blackbird | *Agelaius phoeniceus* | 0 | 8 | 0 | 2 | 0 | 1 (0/0) | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Song sparrow | *Melospiza melodia* | 1 | 1 | 1 | 1 | 1 (0/0) | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Swainson's thrush | *Catharus ustulatus* | 20 | 30 | 1 | 8 | 1 (0/0) | 7 (1/1) | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Swamp sparrow | *Melospiza georgiana* | 0 | 5 | 0 | 1 | 0 | 1 (0/0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Veery thrush | *Catharus fuscescens* | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| White-crowned sparrow | *Zonotrichia leucophrys* | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Wood thrush | *Hylocichla mustelina* | 0 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| **TOTAL** | **13 species (2016) 21 species (2017)** | **174** | **234** | **22** | **57** | **19 (1/2)** | **34 (1/4)** | **3** | **1** | **1** | **19** | **2** | **1** | **1** |

[a] All *Ixodes scapularis* ticks were PCR negative for *Babesia microti* and *Borrelia miyamotoi*.
[b] The Carolina wren is a winter resident at LPBO (i.e., non-migratory). Some birds carried more than one tick species.

**Table 2**
*Ixodes scapularis* ticks collected from Long Point Bird Observatory [LPBO], Point Pelee National Park [PPNP], and Toronto Zoo [TZ] in 2016 and 2017 and results of PCR testing for selected pathogens[a]. Ticks collected from the same environmental sampling location on the same date were pooled by life stage for testing.

| | | | No. of *Ixodes scapularis* PCR-positive for select pathogens[b] | | |
|---|---|---|---|---|---|
| Date collected | Location | No. of *I. scapularis* and life stage[c] | *Babesia odocoilei* | *Anaplasma phagocytophilum* | *Borrelia burgdorferi* s.s. |
| May 2016 | LPBO | 4 A | 0 | 0 | 0 |
| May 2016 | TZ | 3 A | 0 | 1 A | 0 |
| Oct 2016 | TZ | 3 L; 1 N; 4 A | 1 L | 0 | 0 |
| Nov 2016 | PPNP | 41 A | 0 | 0 | 2 |
| May-June 2017 | LPBO | 45 A | 2 A | 0 | 2 A |
| June 2017 | TZ | 24 A | 0 | 0 | 1 A |
| Aug 2017 | TZ | 126 L | 1 A | 0 | 0 |

[a] Ticks collected but not tested for pathogens were *Haemaphysalis leporispalustris* (1 N, LPBO, May 2017), *Dermacentor variabilis* (16 A, LPBO, May 2016; 89 A, LPBO, May-June 2017), *Ixodes cookei* (3 A, PPNP, Nov 2016; 1 N, LPBO, June 2017), *Ixodes marxi* (3 A, PPNP, Nov 2016; 1 N, LPBO, June 2017).
[b] All *Ixodes scapularis* ticks were PCR negative for *Babesia microti* and *Borrelia miyamotoi*.
[c] L = Larva; N = Nymph; A = Adult.

*odocoilei*-associated cervid babesiosis amongst reindeer and wapiti at the TZ in 2012–2015 (Mathieu et al., 2018). Our findings represent the first detections of *Ba. odocoilei* in *I. scapularis* ticks in Ontario, both in questing ticks at the TZ and in the endemic *I. scapularis* population at LPBO, as well as in ticks found on northward migrating birds sampled at LPBO. The establishment of a wildlife reservoir for cervid babesiosis in southern Ontario seems likely given the geographic range and large population of wild white-tailed deer, which is the primary vertebrate host for *Ba. odocoilei*.

Comparison of partial 18S rDNA sequences from tick-derived *Ba. odocoilei* in our study revealed that the majority (4/6) isolated had 100% identity with *Ba. odocoilei* 18S rDNA sequences in GenBank from various hosts and geographic locations, including the wildlife reservoir in Texas, USA (Holman et al., 2000) and clinical cases in cervids from the TZ (Mathieu et al., 2018). This suggests that most strains of *Ba. odocoilei* in southern Ontario are similar if not identical to those reported in the United States. However, the sequence derived from a bird-borne tick in our study showed only 99.5% homology with the other southern Ontario tick-derived sequences and publicly available sequences of *Ba. odocoilei* from various cervid hosts (a single nucleotide difference). This could indicate a different strain of *Ba. odocoilei*, and further molecular characterisation of more genetically diverse targets (e.g., mitochondrial CDS regions) would be required to investigate this further (Ogedengbe et al., 2018).

We detected *Ba. odocoilei* in extracts from homogenized, bisected *I. scapularis* ticks found both in the environment and on migratory birds. However, molecular detection of *Ba. odocoilei* DNA in a host-associated tick does not necessarily reflect true tick infection with the pathogen. The source of this DNA could be either the salivary gland of the tick (i.e., indicating that the tick vector was truly infected with *Ba.*

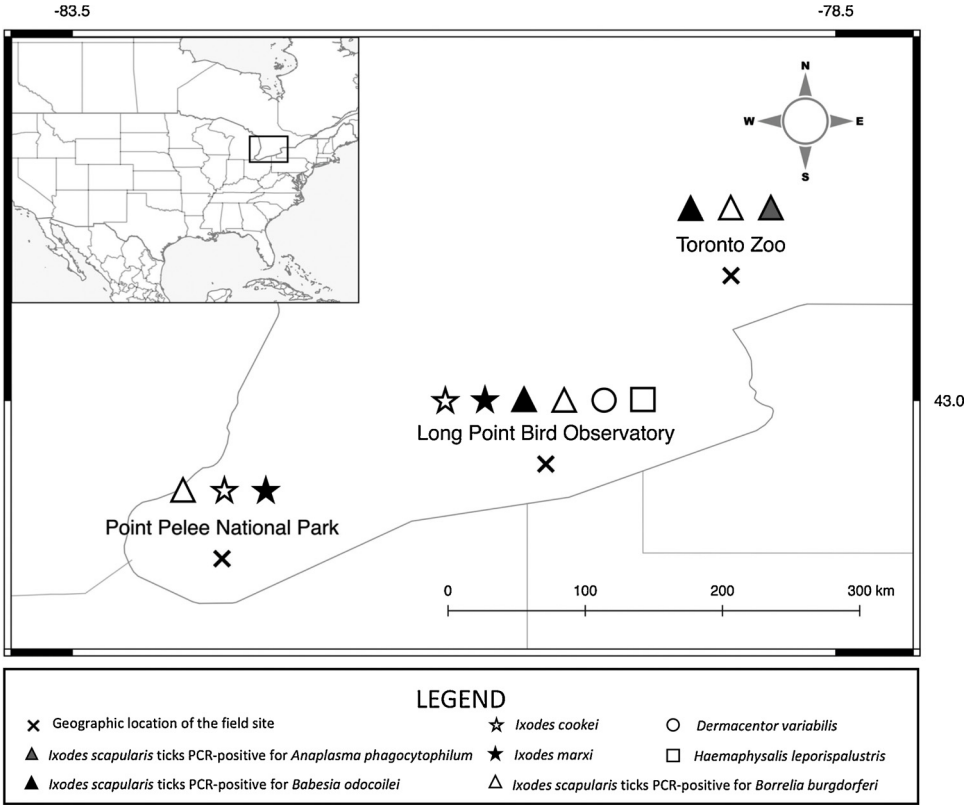


**Fig. 1.** In 2016–2017, questing and host-feeding ticks were collected from three field sites in southern Ontario, Canada, to investigate the infection rate of *Babesia odocoilei, Babesia microti, Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia miyamotoi,* and *Anaplasma phagocytophilum* in *Ixodes scapularis* ticks.

*odocoilei*), or blood in the tick gut derived from recently feeding on an infected vertebrate host (Estrada-Peña et al., 2013). The ability of *Ba. odocoilei* to infect birds and other non-cervid wildlife is unknown. Previous studies have demonstrated that wild birds are competent reservoir hosts for *Ba. microti* (Hersh et al., 2012), so it is possible that our positive results reflect ticks feeding on *Ba. odocoilei*-infected birds or other wildlife. Our finding of *Ba. odocoilei* PCR-positive, unfed, larval tick pools collected using blanket dragging suggest that transovarial transmission of this parasite is possible. This has not yet been demonstrated experimentally for *Ba. odocoilei*, but has been documented for the closely related species, *Babesia divergens*. Transovarial *Babesia* spp. infection may persist over several tick generations, even without new infections derived from blood meals (Uilenberg, 2006). Future studies on the role of *I. scapularis* in the transmission of *Ba. odocoilei* should include blood sampling of avian and other wildlife hosts to survey for *Ba. odocoilei* infection, as well as laboratory studies of vector and reservoir competence.

We surveyed migratory birds to assess whether the transportation of *Ba. odocoilei*-infected ticks over long distances by avian hosts could be a factor in the recent emergence of cervid babesiosis in Canada (Mathieu et al., 2018). In the current study, we found *I. scapularis* on 3.2% of migrating birds captured in spring 2016, and 6.7% in birds captured in 2017. There is an endemic *I. scapularis* population at LPBO that was first documented in the 1970s (Watson and Anderson, 1976), and it is likely that birds acquired ticks at the site of capture as well as carrying ticks to LPBO from the south during their northward spring migration. As did Ogden et al. (2008), we found that the occurrence of *I. scapularis* was highest on ground-foraging bird species such as thrushes. The combined data from both years of our study revealed that 0.2% of birds carried *I. scapularis* ticks that were PCR-positive for *Ba. odocoilei*. Some bird taxa, particularly thrushes, are disproportionately more likely to disperse *I. scapularis* ticks over long distances because they breed in the far northern regions of Canada (Ogden et al., 2015). Larval and nymphal *I. scapularis* feed for 2–4 days on their host, during which time a migratory bird may fly hundreds of kilometres northwards (Marra et al., 2005). In the present study, our finding of a *Ba. odocoilei*-infected larval tick pool attached to a Swainson's thrush illustrates the potential threat of adventitious *Ba. odocoilei*-infected ticks to immunologically naïve wild cervids, such as the boreal population of woodland caribou (*Rangifer tarandus caribou*) found across the northern parts of all Canadian provinces (Thomas and Gray, 2002).

Our study identified *Bo. burgdorferi* s.s. in *I. scapularis* ticks collected from the TZ, LPBO and PPNP, and from migratory birds, consistent with previous reports from southern Ontario (Barker and Lindsay, 2000). Due to logistical constraints, there were marked discrepancies between the seasonal sampling periods for bird-borne and environmental ticks in 2016 and 2017, therefore a direct comparison between years is not possible. Ogden et al. (2008) reported that 18.1% (25/138) of *I. scapularis*-infested birds examined in eastern Canada in 2005–2006 carried at least one *Bo. burgdorferi* s.s.-positive tick, and in 2016–2017, we found that 11.3% (6/53) of *I. scapularis*-infested birds carried at least one *Bo. burgdorferi* s.s.-positive tick. Nymphs of *I. scapularis* are likely to have been infected with *Bo. burgdorferi* s.s. by feeding on a reservoir-competent rodent as larvae (Giardina et al., 2000). However, an alternative explanation for these PCR-positive bird-borne ticks is that the tick gut contained *Bo. burgdorferi* s.s.-infected blood from the avian host. Numerous bird species are competent reservoirs for *Bo. burgdorferi* s.s., including the Swainson's thrush (Scott et al., 2010).

Clow et al. (2017) recently conducted active surveillance of questing *I. scapularis* across 104 sites in Ontario, and did not detect *Ba. microti, Bo. miyamotoi,* or *A. phagocytophilum*. Similarly, we did not identify *Ba. microti* or *Bo. miyamotoi* in any ticks in our study. No detection of *A. phagocytophilum* in bird-borne larvae is not surprising, because this pathogen is not transmitted transovarially. The small sample size of our study likely limited our ability to detect these pathogens, all of which are present but at a low rate of infection (approximately 0.3%) in *I. scapularis* in Ontario (Dibernardo et al., 2014; Nelder et al., 2014). We did detect *A. phagocytophilum* in host-feeding *I. scapularis* adults collected from a wild white-tailed deer carcass at the TZ, giving a minimum infection rate for *A. phagocytophilum* of 0.6% in *I. scapularis* at this location, which is consistent with previous estimates of its prevalence in Ontario (Nelder et al., 2014). *Anaplasma phagocytophilum*-associated clinical disease is an emerging disease of humans (human granulocytic anaplasmosis) and domestic horses (equine granulocytic ehrlichiosis) in the United States (Dumler et al., 2005). Equine granulocytic ehrlichiosis is caused by the same pathogen and was recently reported in a captive herd of Przewalski's horses (*Equus przewalskii*) at a zoo in the mid-Atlantic United States (Sim et al., 2017). The herd of Przewalski's horses housed at the TZ should be considered at high risk for this disease.

In conclusion, we investigated the role of bird-borne and environmental *I. scapularis* ticks in the eco-epidemiology of *Ba. odocoilei, Ba. microti, Bo. burgdorferi* s.s., *Bo. miyamotoi*, and *A. phagocytophilum* in southern Ontario, Canada. Our study provides the first evidence of established local populations of *I. scapularis* ticks in southern Ontario that are infected with *Ba. odocoilei*. We found that *I. scapularis* ticks that test PCR positive for *Ba. odocoilei* are harbored by migratory birds, providing a possible route by which *Ba. odocoilei* could be introduced to naïve cervid populations. The observations reported herein are consistent with and may explain previous detections of the causative agents of Lyme borreliosis and granulocytic anaplasmosis in *I. scapularis* in Ontario. Our study highlights the need for continued public health and veterinary preventative health measures for zoo animals, members of the public, and zoo staff.

## Declarations of interest

None.

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