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RESEARCH ARTICLE

Molecular Detection of *Anaplasma phagocytophilum*, *Babesia odocoilei*, *Babesia species* and *Borrelia burgdorferi* Sensu Lato in Songbirds

John D Scott[1]*, Elena McGoey[2], Ana Morales[3] and Risa R Pesapane[2,4]

[1]Upper Grand Tick Centre, 365 St. David Street South, Fergus, Ontario, N1M 2L7, Canada
[2]School of Environmental and Natural Resources, College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, OH 43210, USA
[3]McGill Bird Observatory, Ste Anne de Bellevue, QC, Canada H9X 0A6
[4]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, 1920 Coffey Rd., Columbus, OH 43210, USA

ABSTRACT

The blacklegged tick, *Ixodes scapularis*, is known to carry various tick-borne zoonotic pathogens with the potential to cause debilitating human and animal diseases. Juvenile *I. scapularis* parasitize songbirds and, perhaps, these avifauna are competent hosts of common microbial pathogens. We extracted brachial venous blood from 18 ground-foraging passerine birds that were parasitized by *I. scapularis* larvae and nymphs. Using molecular identification, namely PCR, DNA sequencing, and Basic Local Alignment Search Tool (BLAST), we targeted *Anaplasma phagocytophilum*, *Babesia* spp. and *Borrelia burgdorferi* sensu lato. Overall, 15 (83%) of 18 passerine birds were positive for 3 microbial zoonotic pathogens that comprised of *A. phagocytophilum* (*n* = 8), *Babesia odocoilei* (*n* = 6), *Babesia* spp. 20-5A74 (*n* = 1), and *B. burgdorferi* sensu lato (*n* = 9). The pathogen load consisted of 8 singles, 5 doubles, and 2 triples. One novel *Babesia* sp. (*Babesia* spp. 20-5A74) was found, and the remaining *Babesia* infections were *B. odocoilei*. Our findings reveal that ground-foraging, passerine birds are avian hosts of zoonotic pathogens. We provide the first-ever documentation that songbirds are hosts of *B. odocoilei*. Based on our data, *B. odocoilei* outnumbered other *Babesia* spp., and elucidated the authentic fact that *B. odocoilei* is the predominant *Babesia* sp. in North America. As avian hosts, passerine birds play a significant role in the enzootic transmission cycle of *B. burgdorferi* sensu lato, *A. phagocytophilum*, and *Babesia* species.

Introduction

Principal vectors of zoonotic pathogens in North America are the blacklegged tick, *Ixodes scapularis* (Acari: Ixodidae), and the western blacklegged tick, *Ixodes pacificus* [1]. Both larvae and nymphs of these ixodid ticks parasitize songbirds (Order: Passeriformes). Epidemiologically, *I. scapularis* carries and transmits at least six tick-borne, zoonotic pathogens that include several genospecies within the *Borrelia burgdorferi* sensu lato (Bbsl) complex [2,3], *Borrelia miyamotoi* [4,5], *Babesia* spp. (Bspp) [6-8], *Anaplasma phagocytophilum* (Aph) [9,10], *Ehrlichia muris eauclairensis* [11], and the virus of Powassan Virus Disease [12-14]. Ecologically, *I.*

***Corresponding author(s)**

John D Scott, Upper Grand Tick Centre, 365 St. David Street South, Fergus, Ontario N1M 2L7, Canada

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scapularis parasitizes at least 82 bird species [15] and 55 mammalian hosts, including humans [7,16-21]. Polymicrobial infections have been reported in patients [22], and multiple pathogens (i.e., Bbsl, *B. miyamotoi*, *A. phagocytophilum*, *B. microti*) have been detected in a single *I. scapularis* [23].

The Lyme disease bacterium was first isolated from the blood of Common Yellowthroat (*Geothlypis trichas*), American Robin (*Turdus migratorius*), and Gray Catbird (*Dumetella carolinensis*) collected in Connecticut [16]. As well, Bbsl was cultured from a Song Sparrow (*Melospiza melodia*) collected in Wisconsin [24] and, likewise, in Connecticut [16-18]. In Canada, Bbsl-positive *I. scapularis* larvae have been collected from Song Sparrows [25,26]. Researchers put xenodiagnostic larvae on American Robins, and determined that this avian host is a competent host of Bbsl [27]. *Anaplasma phagocytophilum* has been detected in the blood of birds collected in California [28]. Specifically, blood samples from a Golden-crowned Sparrow (*Zonotrichia atricapilla*) and European Starling (*Sturnus vulgaris*) were positive for *A. phagocytophilum*.

Worldwide, several different *Babesia* spp. (Apicomplexa: Piroplasmida: Babesiidae) cause human babesiosis, and they include *B. crassa*-like [29], *B. divergens* [30], *Babesia divergens*-like MO-1 [31], *B. duncani* [32], *B. microti* [33], *B. motasi* [34], *B. odocoilei* [7], *Babesia* spp. XXB/HangZhou [35], *Babesia* sp. TW1 [36], *Babesia* spp. CA1, CA3, and CA4 [37], and *B. venatorum* [38]. Also, at least 111 valid *Babesia* species are present in environmental habitats around the globe [39]. Using molecular-based characterization, molecular biologists and veterinary researchers recently discovered *Babesia odocoilei* (Bod) in red deer, *Cervus elaphus*, in the United Kingdom [40].

Migratory songbirds widely disperse *I. scapularis* larvae and nymphs infected with tick-borne, zoonotic pathogens, including *B. odocoilei*. Combining datasets, *B. odocoilei* is common in North America. In the USA, tick researchers have reported *B. odocoilei* in Indiana [41-43], Michigan [44] Maine [42,43], Massachusetts [41-43], New York [45], Oklahoma [46,47], Pennsylvania [48,49] Texas [50,51], Virginia [52], and Wisconsin [42,43]. As well, *B. odocoilei* has been detected in *I. pacificus* in California [53]. In Canada, *B. odocoilei* has been detected in Saskatchewan [54], Ontario [7,15,55-59], and Quebec [55,57,58]. And yet, acarologists and ecologists have not reported *B. microti* in these three provinces [7,15,21,55-59].

Babesia odocoilei, which is a sequestering *Babesia* sp., can be recalcitrant to treat in human patients [7]. In contrast, *B. microti* is a non-sequestering species, and is relatively easy to treat. Biogeographically, there is paucity of *B. microti* continentwide [55].

The purpose of this tick-host-pathogen study was to take blood samples from ground-foraging songbirds that were parasitized by blacklegged ticks, *I. scapularis*, and determine whether passerine birds are hosts *A. phagocytophilum*, *Babesia* spp., and *B. burgdorferi* sensu lato.

Materials and Methods

Bird blood collection

Songbirds were captured using standard mist nets in two different locations: an arboreal area near Montée Biggar, Quebec, Canada; 45.09 N, 74.22 W and, also, McGill Bird Observatory, Ste-Anne-de-Bellevue, Quebec; 45.43 N, 73.94 W.

Blood (~50 µL) was collected in non-heparinized microhematocrit capillary tubes (Fisherbrand™, Saint-Laurent, Quebec, Canada) from the brachial vein of each bird, by trained individuals, between 16 June 2021 to 25 August 2021. The blood was then transferred to 1.5 mL cryogenic tubes (Thermo Fisher Scientific™, Mississauga, Canada; Nalgene™, Singapore, Southeast Asia) filled with 0.5 mL Queen's lysis buffer solution [60]. Samples were stored inside a fridge for up to 6 months at 4°C until they were shipped to the lab for subsequent analysis. Banding and sampling of songbirds were granted under animal use protocol 2007-5446 for McGill University, and federal banding permits were issued by the Canadian Wildlife Service.

DNA isolation and pathogen detection

Genomic DNA was extracted from songbird blood using the PureLink Genomic DNA Mini Kit (Invitrogen, Waltham, MA, USA) according to manufacture's instructions. Detection of *B. burgdorferi* s.l. and *A. phagocytophilum* was performed using 20 µL real-time PCR reaction of Taqman Fast Advanced Master Mix (Applied Biosystems, Waltham, MA. USA) and previously established primers targeting the 16S rDNA and *msp2* genes, respectively [61,62]. A cycle threshold ≤ 40 with a characteristic curve was considered positive, and all positives were run in duplicate. Detection of *Babesia* spp. was achieved using primers targeting the 18S gene in conventional PCR followed by sequencing as previously described [15,58]. Molecular biology grade water and synthetic gBlock gene fragments (Integrated DNA Technologies, Coralville, IA, USA) of *B. burgdorferi*

(MH781147), *A. phagocytophilum* (AY151054.1) and *B. microti* (MT974173.1) were included in all PCR reactions as controls.

Results

Tick collection

In total, brachial venous bloods from 18 ground-forging passerine birds (6 Song Sparrows, *Melospiza melodia* Wilson; 4 Common Yellowthroats, *Geothlypis trichas* L.; 4 Veeries, *Catharus fuscescens* Stephens; 1 Ovenbird, *Seiurus aurocapillus* L.; 1 Brown-headed Cowbird, *Molothrus ater* Boddaert; 1 Swainson's Thrush, *Catharus ustulatus* Nuttall; and 1 American Robin, *Turdus migratorius* L.) were selected for testing and analysis. Qualified bird banders identified passerine birds to bird species. From these tick-infested passerines, 30 *I. scapularis* (9 larvae, 21 nymphs) were also collected.

Molecular detection of pathogens

Overall, 24 infections were detected, namely 9 Bbsl (38%), 8 Aph (33%), 6 *B. odocoilei* (25%), and one *Babesia* spp. 20-5A74 (4%), in 15 (83%) of 18 songbirds. These infections comprised of 8 single infections, 5 co-infections (n = 10), and 2 polymicrobial infections (n = 6) (Table 1).

Nine birds (50%) were infected with Bbsl, six birds (33%) with *B. odocoilei*, and eight birds (44%) with Aph. A single bird (Common Yellowthroat) was infected with a *Babesia* spp. 20-5A74 strain (Table 1).

Blood from four different Song Sparrows were positive for Bbsl which also suggests that Song Sparrows are avian hosts of Bbsl. One (SOSP*0976) of these four Song Sparrows had a triple infection (i.e., Bbsl, Aph, Bod) (Figure 1A). Likewise, a juvenile Brown-headed Cowbird (BHCO*1471) had three endogenous pathogens (i.e., Bbsl, Aph, Bod) simultaneously as a polymicrobial infection (Figure 1B). Three individual songbirds (1 Common Yellowthroat, 1 Veery, and 1 Song Sparrow) were not infected with target pathogens.

Since the seven *Babesia* sequences for *B. odocoilei* were all almost 99.7% or more identical to sequences in GenBank, we did not deposit them to this publically available databank because they would not contribute to known diversity.

Discussion

The tick *I. scapularis* carries various pathogens with the potential of producing serious human and animal diseases. *Babesia odocoilei*-infected *I. scapularis* ticks have previously been collected from songbirds, but we unveil this babesial parasite for the first time in bird blood. After numerous *B. odocoilei* collections in Ontario and Quebec, *B. microti* has not been found in blacklegged ticks or songbirds. Not only do ground-frequenting songbirds transport ticks, they may also be hosts for tick-borne, zoonotic pathogens. Migratory songbirds widely disperse zoonotic pathogens across North America and, therefore, one does not have to frequent or live in an endemic area to contract human babesiosis caused by *B. odocoilei*.

Tick-Host-Pathogen enzootic transmission cycle

Each microbial pathogen in the present study has its own tick-host pathways for sustaining viability. For polymicrobial infections, each one can have a different source and enzootic route. Tick-borne, zoonotic pathogens can be sourced from ixodid ticks, and transmitted during bird parasitism [15,55-59]. Since *I. scapularis* ticks are typically infected, we sampled ground-foraging songbirds with attached ticks. Although we selected these parameters, there was no assurance of infectivity. Depending on the time of year, sources and pathways of pathogens can vary greatly. Moreover, the combination of pathogens, can vary in the enzootic transmission cycle.

Table 1. DNA sequence analysis of the 18S gene of *Babesia* spp. in blood of songbirds captured at Montée Biggar, Quebec, 2021.

Bird Species	Bird Code	Date Collected	*Babesia* species	BLAST Match
Common Yellowthroat	COYE*2978	12-Jul	*B. odocoilei*	99.8/99
Common Yellowthroat	COYE*7109	16-Jun	*B.* spp. 20-5A74	94/96
Common Yellowthroat	COYE*7128	23-Jun	*B. odocoilei*	98.8/100
Veery	VEER*1549	12-Jul	*B. odocoilei*	100/100
Veery	VEER*3257	16-Jun	*B. odocoilei*	99.2/100
Song Sparrow	SOSP*0976	4-Jul	*B. odocoilei*	99/100
Brown-headed Cowbird	BHCO*1471	4-Jul	*B. odocoilei*	98.9/100

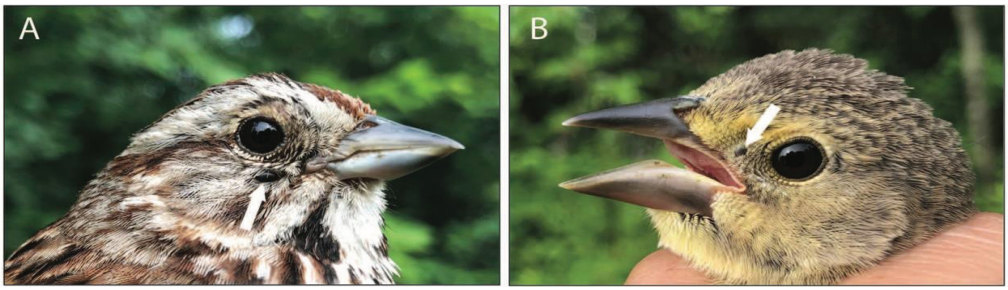

Figure 1. Songbirds with triple infections comprised of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, and *Babesia odocoilei*. **A.** Song Sparrow, SOSP*0976, adult; **B.** Brown-headed Cowbird, BHCO*1471, juvenile. White arrows point to engorged, attached ticks. Photos: Ana Morales.

Even though *I. scapularis* females do not parasitize songbirds, they can transmit *B. odocoilei* to the next generation via transovarial transmission (gravid female to eggs to larvae) [1,46,63]. When larvae acquire *B. odocoilei*, they can transmit *B. odocoilei* to the next life stages via transstadial passage (larva to nymph and/or nymph to adult). Notably, neither larvae nor nymphs require a *B. odocoilei*-infected host to acquire infection. If the mobile life stages (i.e., larvae, nymphs, and females) of *I. scapularis* do not imbibe a replete blood meal, transmission of *B. odocoilei* is discontinued.

Alternatively, *I. scapularis* must acquire *B. burgdorferi* s.l. and *A. phagocytophilum* from infected hosts. Both bacterial infections typically stop when *I. scapularis* adults die. However, ground-foraging songbirds often feed on spent females after she lays her eggs, and may become infected orally. Hosts are paramount in sustaining these bacterial infections [63].

Although inter-generational studies have not been done with passerines, they can hold microbial infections as competent hosts [27]. Four (80%) of five Song Sparrows were infected with *B. burgdorferi* s.l. which suggest this bird species is a competent host. As well, three (75%) of four Common Yellowthroats were infected with *A. phagocytophilum* suggesting they are competent hosts. Similarly, three (75%) of the four Common Yellowthroats were infected with *Babesia* spp. Furthermore, two (50%) of four Veeries were infected with *B. odocoilei* which, again, suggests that Veeries are competent hosts of this apicomplexan parasite.

Polymicrobial infections occur in *I. scapularis* and their hosts. In the present study, an adult Song Sparrow (SOSP*0976) was infected with *A. phagocytophilum*, *B. odocoilei*, and *B. burgdorferi* s.l.; however, the single *I. scapularis* nymph was negative. Likewise, a juvenile Brown-headed Cowbird (BHCO*1471) was infected simultaneously with these three tick-borne zoonotic pathogens; however, the engorged larvae, which molted via transstadial passage to nymphs, were negative. These enzootic findings suggest vertical transmission (mother songbird to filial offspring) or previous infections via bird parasitism by ticks. When juvenile *I. scapularis* are negative, and the hosts' bloods are positive, this enzootic situation suggests that these avian hosts are retaining tick-borne zoonotic pathogens in their bodies, and are competent hosts.

With respect to *B. burgdorferi* s.l., *I. scapularis* females do not facilitate transovarial transmission [62]. Therefore, when an *I. scapularis* larva parasitizes a songbird, the only avenue for it to become infected with *B. burgdorferi* s.l. is an infected host (Figure 2). Birds are known to harbor Bbsl in their bodies for extended periods of time [27,64,65]. Once acquired from infected hosts, many tick-borne pathogens are confined within the tick gut lumen, and are surrounded by tick-microbe interactions and discrete midgut barriers [66]. There are many barriers that pathogens encounter when passing from host to tick. These biological hindrances include host blood chemistry, tick defenses, competition from other pathogens/microbes within the tick, and sequence of infection (e.g., some pathogens may exclude others depending on the order of exposure) [66-69]. A tick can initially test positive for a microorganism but not survive due to tick characteristics and microbial interactions in the midgut lumen.

Overall, *B. microti* is sparse in North America. After numerous tick-host-pathogen studies, researchers have not found *B. microti* in *I. scapularis* and songbirds collected in Ontario and Quebec [7,8,15,21,55-59]. Similarly, a tick-pathogen survey of 299 questing *I. scapularis* adults in Pennsylvania found *B. odocoilei*

Figure 2. Veery, VEER*1207, hatch year, parasitized by three *Ixodes scapularis* larvae. These replete larvae molted to unfed nymphs. This bird was infected with *A. phagocytophilum* and *B. burgdorferi* s.l.; however, the attached larvae were absent of the three pathogens tested. Photo: Ana Morales.

in 15.4%, whereas *B. microti* accounted for only 0.7% [49]. In Maryland, none of the 348 *I. scapularis* nymphs collected was positive for *B. microti* [70]. In a recent tick-host-pathogen study, the ratio of *B. odocoilei* to *B. microti* was 41 to 1 [55]. By far, *B. odocoilei* was the predominant *Babesia* sp. With the exception of one *Babesia* strain (i.e., *Babesia* spp. 21-5A74), all *Babesia* spp. in the present study were *B. odocoilei* (Table 1). Moreover, since most commercial laboratories do not test humans for *B. odocoilei*, and the fact that *B. odocoilei* cross-reacts with *B. duncani* [7,8], *B. odocoilei* has often been misrepresented or discounted as *B. duncani* [7,8].

Survival of *Babesia odocoilei* in nature

Over millions of years, *B. odocoilei* has honed itself to parasitize a wide range of vertebrate hosts [38,63]. This sequestering piroplasmid can dwell in all four developmental life stages (eggs, larvae, nymphs, adults) of *I. scapularis* ticks [1,59]. *Babesia odocoilei*-infected, gravid females can pass this infection to offspring via transovarial transmission [1,46,63]. From the midgut epithelium, kinetes (infective babesial spores) move through the tick body fluid (haemolymph) to peripheral tissues, including the ovaries. After mating, the eggs become infected with *B. odocoilei* kinetes, and are ready to be deposited on the forest floor. After 5-6 wk, the clutch of eggs hatch to *I. scapularis* larvae, and are ready to transmit *B. odocoilei* to suitable hosts. As well, larvae can transmit *B. odocoilei* infection to hosts by transstadial passage [1,46,63]. In fact, a *B. odocoilei*-infected *I. scapularis* female can transmit *B. odocoilei* infection from one tick generation to the next generation without parasitizing infected hosts. In nature, this generational sequel completes an enzootic transmission cycle of *B. odocoilei*. Because of their minute size (0.75 mm), and capability to transmit *B. odocoilei*, *I. scapularis* larvae pose a substantial threat to the local human population, especially after mid-July when a clutch of eggs hatch to larvae. Protective acaricide-treated clothing is important, especially when the temperatures are above freezing and there is no snow cover. Not only are white-tailed deer, *Odocoileus virginianus*, hosts of all three host-seeking life stages (larvae, nymphs, adults) of *I. scapularis*, these cervids are reservoirs of *B. odocoilei* [46,71]. Based on our findings, songbirds not only transport larval and nymphal *I. scapularis*, they are competent hosts of at least three tick-borne zoonotic microorganisms.

The blood sample of a Common Yellowthroat (COYE*7109) was confirmed positive for the apicomplexan species, *Babesia* spp. 20-5A74. This novel strain was likewise detected in an *I. scapularis* female parasitizing a domestic cat residing in the western part of eastern Ontario [15]. Ecologically our findings show that passerine birds can harbor polymicrobial infections including *A. phagocytophilum*, *B. odocoilei*, *Babesia* spp. 20-5A74 and *B. burgdorferi* s.l.

Differences in *Babesia* species

Babesia odocoilei has special adaptations to live in ixodid ticks and certain avian and mammalian hosts, including humans. Pathologically, *B. odocoilei* causes human babesiosis [7,8], and ecologically, all four life stages of *I. scapularis* can harbor this zoonotic infection [1,63]. In mammalian hosts, sequestering *Babesia* spp., such *B. odocoilei* maintain infection by using cytoadherence (adheres to endothelium cells and lining) [72]. Additionally, *B. odocoilei* exhibits sequestration (intravascular *Babesia* entanglements consisting of uninfected- and infected-erythrocytes bonded by fibrin strands), and occlude and block capillaries and post-capillary venules [73,74]. Sequestering *Babesia* spp. can complete their life cycle within self-perpetuating entanglements (local proliferation), and cause febrile symptoms. Thus, they remain isolated from the circulating immune system and spleen [74]. Compared to non-sequestering *Babesia* species (i.e., *B. microti*), patients that are infected with *B. odocoilei* are typically recalcitrant to treat with standard anti-*Babesia* regimens [7,8]. Patients may become life-long carriers and, occasionally, death results [31,75]. Sequestration has been documented in other *Babesia* spp. including *Babesia bovis* in cattle [72], *Babesia canis* in dogs [76],

Babesia lengau in domestic cats [77]. In contrast, other *Babesia* spp., which are non-sequestering, include *B. bigemina*, *B. divergens*, and *B. microti* [78]. Since there at least 111 valid *Babesia* species, more yet-to-be-recognized, sequestering *Babesia* species will be flourishing in indigenous areas around the globe.

Human babesiosis caused by *Babesia odocoilei*

Human babesiosis caused by *B. odocoilei* is now being diagnosed and treated clinically [7,8]. Presentation in human patients varies from asymptomatic to debilitating with variable circulatory, gastrointestinal, rheumatological and neurological manifestations. Symptoms typically include fatigue, exertional intolerance, inflammation, ischemia, impaired cognition, cold intolerance, digital numbness, sweats (especially at night), insomnia, tissue/organ dysfunction, muscle aches (especially legs), and loss of balance [7,8]. Since *B. odocoilei* sequesters in the capillaries of the brain, this intraerythrocytic infection can produce cerebral pathophysiology, coma-like symptomology, and brain fog. Self-perpetuating, fibrin-bonded entanglements cause occlusions in capillaries, and hinder blood circulation. These entanglements induce capillary blockage and, thus, impede the transfer of oxygen and nutrients. Consequently, mitochondria are forced to produce ATP anaerobically and, therefore, these minute organelles operate inefficiently producing excess lactic acid. Diminutive ATP exists for normal cellular functions, including the Na^+/K^+ pump [79]. Mitochondria dysfunction and occlusion of capillaries associated with sequestering *Babesia* spp. help to explain the symptoms of human babesiosis caused by *B. odocoilei*. Humans with a sequestering babesial infection can have cognitive and mood disorders ranging from minimal to severe [80]. *Babesia odocoilei* infections are typically persistent because this piroplasmid sequesters in capillaries and venules. Once this infection develops to the advanced stage, this deep-seated, stealth infection is recalcitrant to treat with current anti-*Babesia* regimes and, thus, this babesial infection is chronic [7,8]. Since there has not been a valid commercial test for *B. odocoilei* aimed at human subjects, this particular human babesiosis has been a longstanding issue (i.e., cross-reactions) that has been misrepresented by invalid serology tests and unsubstantiated medical evaluations [1,55]. Tick bite is the normal mode of transmission, but blood transfusion is also apparent. Case reports of congenital babesiosis have been documented [81-83]. Infants can acquire babesiosis from tick bites, blood transfusions, or congenitally via vertical transmission (mother to filial offspring). Babesiosis in neonates can present with febrile thrombocytopenia, fevers, and parasitemia.

Conclusion

We provide the first documentation of songbirds as hosts of *B. odocoilei*, namely Song Sparrows, Common Yellowthroats, and Veeries. Since transovarial transmission does not apply to *B. burgdorferi* s.l. and *A. phagocytophilum*, we provide substantive evidence that Song Sparrow and Common Yellowthroats are competent hosts of these two zoonotic pathogens. Because *B. odocoilei* exhibits transovarial transmission, we also show supportive evidence that Common Yellowthroats are competent hosts of *B. odocoilei*. Based on our data, *B. odocoilei* outnumbers other *Babesia* species, and elucidates its predominance within the Temperate Zone of North America. In addition, we show that songbirds can harbor at least three different pathogens concurrently. Molecular analysis yielded *A. phagocytophilum*, *B. odocoilei*, *Babesia* spp. 20-5A74, and *Borrelia burgdorferi* s.l. in the blood of songbirds captured in southern Canada. As avian hosts, passerines play a noteworthy role in the enzootic transmission cycle of *B. burgdorferi* s.l., *A. phagocytophilum* and *Babesia* spp. Unequivocally, *B. odocoilei* is the predominant *Babesia* spp. in North America. Because songbirds transport pathogen-laden *I. scapularis* throughout North America, outdoors people do not have to live in or visit an endemic area to contract tick-borne, zoonotic pathogens. Healthcare practitioners must be cognizant that patients with a tick bite may have acquired polymicrobial infections.

Acknowledgments

Ethical consideration

Ethical approval for banding and sampling of songbirds were granted under animal use protocol 2007-5446 for McGill University, and federal banding permits issued by the Canadian Wildlife Service.

Authors' contributions

Conceptualization and design: JDS and RRP. Collection and methodology: JDS, AM, EM and RRP. Formal analysis: JDS, EM, and RRP. Drafting of manuscript: JDS, EM, and RRP. Accuracy of data analysis: JDS, RRP. All authors read and approved the final version of the manuscript.

Competing financial and investment interests

The authors declare that they have no competing

financial and investment interests related to these tick-borne zoonotic studies.

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Recognition

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