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RESEARCH ARTICLE

Molecular detection of *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi*, *Babesia odocoilei*, *Babesia microti* and *Anaplasma phagocytophilum* in *Ixodes* ticks collected across Canada

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Abstract

Tick-borne zoonotic diseases are a profound challenge to healthcare practitioners, and an overwhelming scourge to patients worldwide. On the whole, patients have great difficulty getting diagnosed and treated, and often become chronically ill. In this study, we tested 224 ticks consisting of *Ixodes angustus*, *Ixodes pacificus*, and *Ixodes scapularis*. Using real-time PCR and nested PCR, we obtained the following positives: *Borrelia burgdorferi* sensu lato (*n* = 74), *Borrelia miyamotoi* (*n* = 4), *Babesia odocoilei* (*n* = 82), *Babesia microti* (*n* = 1), and *Anaplasma phagocytophilum* (*n* = 8). Markedly, *B. odocoilei* and *B. burgdorferi* were detected in *I. scapularis* ticks nationwide. As well, the Canada-wide prevalence of *B. burgdorferi* s.l. and *B. odocoilei* in *I. scapularis* adults was 40% and 36%, respectively. The statistical ratio of *B. odocoilei* to *B. microti* in *I. scapularis* adults was 60 to 1. *Babesia odocoilei* is, unquestionably, the predominant *Babesia* sp. across Canada. We provide the first report of *B. odocoilei* in an *I. angustus* tick. In addition, we unfurl the first report of *B. odocoilei* in *I. scapularis* in British Columbia, Alberta, Saskatchewan, Manitoba, Prince Edward Island, and Newfoundland and Labrador. From a professional healthcare standpoint, *I. scapularis* ticks are just as likely to be infected with *Babesia odocoilei* as *Borrelia burgdorferi* s.l. Since people spend considerable time in outdoor areas, clinicians must be familiar with current acumen in tick-borne zoonotic diseases.

Introduction

Tick-borne zoonotic diseases inflict immense medical, veterinary, and economic losses globally. *Ixodes angustus*, the small mammal tick (Acari: Ixodidae), the western blacklegged tick,

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Ixodes pacificus, and the blacklegged tick, *Ixodes scapularis* are involved in transmitting tick-borne zoonotic pathogens in North America [1]. Recently, researchers documented *I. scapularis* in British Columbia (BC), so this tick species is nationwide [2]. Both *I. pacificus* and *I. scapularis*, are the primary vectors of several tick-borne zoonotic pathogens. In fact, *I. scapularis* carries and transmits at least six tick-borne zoonotic pathogens, namely genospecies of *Borrelia burgdorferi* sensu lato (Bbsl) complex [3,4], *Babesia* spp. (*B.* spp.) [5,6], *Anaplasma phagocytophilum* (Aph) [7,8], *Borrelia miyamotoi* [9], *Ehrlichia muris eauclairensis* [10], and the virus of Powassan Virus Disease [11,12].

Left untreated or inadequately treated, tick-borne diseases become chronic and, in the case of Lyme disease, is considered chronic at the 6-month mark [13,14]. Recently, Bbsl, *Babesia odocoilei* (*B. odocoilei*), and *A. phagocytophilum* have been reported in *I. pacificus* in BC [15].

Babesia odocoilei (Apicomplexa : Piroplasmidae: Babesiidae) is a single-celled, red blood cell parasite that is pathogenic to humans [5,6]. This one-celled piroplasmid is widespread in North America, and has been reported in at least 16 states including California [16], Indiana, Maine, Massachusetts [17,18], Michigan, Minnesota, Montana, New Hampshire [19-21], New York [20-22], Ohio [20], Oklahoma [23,24], Pennsylvania [21,25,26], Texas [21,27,28], Virginia [29], Washington [30], and Wisconsin [17,18,20,23,25,31]. In Canada, *B. odocoilei* has been documented in Saskatchewan [32], Nova Scotia, Quebec, Southern Ontario, Northern Ontario [33], and BC [15]. In a former tick-pathogen study, which was conducted in eastern and central Canada, the ratio of *B. odocoilei* to *B. microti* detected in *I. scapularis* adults was found to be 41 to 1. These findings spearheaded a new understanding: *B. odocoilei* is the most prevalent *Babesia* sp. in North America [33].

Globally, there are at least 111 valid *Babesia* spp. [34]. Some of these *Babesia* species are pathogenic to humans, including *B. bengimina* [35] *B. crassa/crassa*-like [36], *B. divergens* [37], *Babesia divergens*-like MO-1 [38], *B. duncani* [39], *B. microti* [40], *B. motasi* [41], *B. odocoilei* [5,6], *Babesia* spp. XXB/HongZhou [42], *Babesia* sp. TW1 [43], *Babesia* spp. CA1, CA3, and CA4 [44], and *B. venatorum* [45].

The primary reservoirs of *B. odocoilei* are cervids (i.e., the white-tailed deer, *Odocoileus virginianus*) [27,28] and Columbia black-tailed deer, *Odocoileus hemionus columbianus* [46]. *Babesia odocoilei* is also infective to desert bighorn sheep, *Ovis canadensis nelsoni* [46]. Historically, the first isolations of *B. odocoilei* were obtained from white-tailed deer in Texas in 1968 [27,28]. More recently, *B. odocoilei* has been detected in songbird-transported *I. scapularis* larvae and nymphs [47-52]. This *Babesia* species has also been detected in *I. scapularis* adults [47-51]. Notably, this intraerythrocytic *Babesia* parasite has been detected in the blood of ground-foraging songbirds [52]. In North America, both *I. pacificus* [15] and *I. scapularis* [47-52] harbor *B. odocoilei*. From an epidemiological standpoint, *B. odocoilei* can infect *I. pacificus* and *I. scapularis*, and can be transmitted to humans and cause human babesiosis [5,6].

In retrospect, Banerjee SN, et al. [53] found Bbsl in 24 locations in BC where people had encounter signs and symptoms of Lyme disease. They detected Bbsl in ticks at each of these sites located in southwestern BC, including the Lower Mainland Region, Metro Vancouver, South and Central Vancouver Island, and off-shore islands in the Salish Sea. Biologically, these researchers detected Bbsl in both ticks and small mammals. Epidemiologically, ticks often harbor tick-borne zoonotic pathogens with polymicrobial infections [54,55]. In fact, four different pathogens have been documented in a single *I. scapularis* adult [54]. Coinfections of polymicrobial pathogens can unknowingly be

transmitted to humans, simultaneously, during a tick bite. When these malignant co-infections go undetected, they can become persistent systemic infections [56]. Using a molecular diagnostic technique, East Coast researchers recently detected *B. odocoilei* in humans residing in Michigan, New Jersey, North Carolina, Oklahoma, and Mexico [57].

The purpose of this study was 1) to gauge the distribution *A. phagocytophilum, B. microti, B. odocoilei, Bbsl,* and *Borrelia miyamotoi* across Canada, 2) to determine the prevalence of these five tick-borne zoonotic pathogens in *I. angustus*, *I. pacificus*, and *I. scapularis*, and 3) assess the effect of *B. odocoilei* on humans.

Materials and Methods

Tick collection

Veterinarians, veterinary technicians, wildlife rehabilitators, bird banders and the public collected ticks from avian and mammalian hosts, and by flagging. With the exception of wildlife rehabilitators, which kept ticks alive, ticks were put in micro tubes containing 94% ethyl alcohol. Specifically, wildlife rehabilitators put ticks in polyethylene, round-bottom tubes capped with a rounded piece of 25 mm X 25 mm tulle netting to enclose the live ticks, thus, keeping them from escaping. A polypropylene stopper, which had a 7-mm hole, allowed the tube to ventilate. These tubes were put in a self-sealing plastic bag containing a slightly moisten paper towel. This bag was put in a bubble pack envelope, and sent to the laboratory for identification (J.D.S.) and, subsequently, for testing. An Olympus SZX16 stereoscopic microscope, and taxonomic keys were used to assist in tick identification [58-60].

Molecular analyses

All DNA extractions and PCRs were completed by Geneticks Inc. Adult ticks were bisected longitudinally, and homogenized using a sterile pestle in 200 µl of DNA/RNA Shield (Zymo Research). Total nucleic acid was extracted from homogenized tick halves using the Quick-DNA/RNA Pathogen Miniprep (Zymo Research) following the manufacturer's instructions.

Real-time PCR (qPCR) and nested PCR (nPCR) assays compared the performance of pathogen detection. The primers and probes are listed in table 1. All samples were tested for the presence of *Borrelia* species, *B. burgdorferi* sensu stricto, *B. miyamotoi*, *A. phagocytophilum*, *B. microti*, and *B. odocoilei*. All *Borrelia* testing was performed using real-time PCR in 30 µl reaction volumes using 15 µl of PCRBIO Probe Blue Mix (PCR Biosystems), 800 nM of both forward and reverse primers, 250 nM of probe, and 10 µl of extracted total nucleic as template. Reactions were subjected to an initial denaturation of 8 min at 95˚C, followed by 40 cycles each of two stages: 1) 95˚C for 10 sec, and then 2) 60˚C for 30 sec. Real-time PCR reactions were performed using an iCycler IQ real-time PCR system (BioRad) according to the manufacturer's instructions.

Detection of *A. phagocytophilum*, *B. microti*, and *B. odocoilei* was performed by nested PCR in 25 µl reaction volumes using 12.5 µl of 2x Taq FroggaMix (Frogga Bio Scientific Solutions), 400 nM of both forward and reverse primers, and 2 ul of template. The outer reaction conditions for *A. phagocytophilum* included an initial denaturation at 95˚C for 10 min, followed by 35 cycles at 95˚C for 30 sec, 53˚C for 30 sec, 72˚C for 1 min, and a single final extension of 72˚C for 10 min. The inner reaction conditions were identical, except annealing was performed at 55˚C, and 40 total reaction cycles were used. The outer reaction conditions for *B. odocoilei* include an initial denaturation at 95˚C for 10 min, followed by 40 cycles at 95˚C for 30 sec, 58˚C for 30 sec, 72˚C for 30 sec, and a single final extension of 72˚C for 10 min. The inner reaction conditions were identical, except annealing was performed at 63˚C for 15 sec, and extension was performed at 72˚C for 20 sec.

Table 1: Pathogen detection primes and probes.

Genus/Species	Gene	PCR Type	Primer name	Sequence (5'-3')	Amplicon Size	References
Borrelia species	23s IGS	qPCR	Bb23Sf	cgagtcttaaaaggggcgatttagt	75	Courtney et al., 2004 [61]
			Bb23Sr	gcttcagcctggccataaatag		
			Bb23SFAM	FAM-agatgtggtagacccgaagccgagtg-ECLIPSE		
Borrelia burgdorferi sensu stricto	opsA	qPCR	ospAF	ccttcaagtactccagatccattg	95	Tokraz et al., 2017 [62]
			ospAR	aacaaagacggcaagtacgatc		
			ospAProbe	FAM-TGCAACAGTAGACAAGCTTGAGCT-ECLIPSE		
Borrelia miyamotoi	flab	qPCR	flaBf	Agcacaagcttcatggacattga	102	
			flaBr	Gagctgcttgagcaccttctc		
			flabProbe	FAM-tgtgggtgcaaatcaggatgaagca-ECLIPSE		
Anaplasma phagocytophilum	Msp2	Nested PCR	AnaP44OutL1-F	GTAGAAGAAACCGCCCTAAT	850	n/a
			AnaP44OutL1-R	TCTATGTTGGTTTGGAATTACAG		
			MSP3F0	CCAGCGTTTAGCAAGATAAGAG	334	Holden et al., 1992 [63]
			MSP3R	GCCCAGTAACAACATCATAAGC		
Babesia microti	18s rRNA	Nested PCR	Babs1	CTTAGTATAAGCTTTTATACAGC	238	Persing et al., 1992 [64]
			Bab4	ATAGGTCAGAAACTTGAATGATACA		
			Bab2	GTTATAGTTTATTTGATGTTC	155	
			Bab3	AAGCCATGCGATTCGCTAAT		
Babsia odocoilei	18s rRNA	Nested PCR	Bab306R_RCF	TTTCTGCGTCACCGTATT	331	Burgess et al., 2021 [65]
			BabGenInR2	ACGACGGTATCTGATCGTCT		
			Odo563	CCGTATTTTGACTTTTGTCGACTGT	311	N/A
			BabGenInR1	TCTGATCGTCTTCGATCCCC		

Both outer and inner reaction conditions for *B. microti* included an initial denaturation at 95˚C for 10 min, followed by 35 cycles at 95˚C for 30 sec, 55˚C for 30 sec, 72˚C for 30 sec, and a single final extension at 72˚C for 10 min. All nested PCR reactions were performed in a MJ Research PTC-225 Tetrad Thermocycler (BioRad) according to manufacture's instructions.

Results

Tick collection

In total, 224 *Ixodes* ticks (Table 2) consisting of *I. angustus*, *I. pacificus*, and *I. scapularis* were tested using nested PCR because this molecular PCR technique gave the most prodigious yield. The nested PCR outperformed the single-step *Babesia* primer (qPCR) and, thus, we employed nested PCR for our final, summary results.

Of the juvenile ticks collected from songbirds, four live ticks molted to the next life stage. Geographically, 10 ticks were collected from avian and mammalian hosts in BC (Table 3), but not tested for tick-borne pathogens because they are not known to bite humans.

Of note, 72 (40%) of 180 *I. scapularis* adults collected across Canada yielded a significant prevalence for *B. burgdorferi* sensu lato. Similarly, the prevalence of *B. odocoilei* in *I. scapularis* adults collected Canada-wide was 65 (36%) of 180 collected.

Polymicrobial infections consisting of double infections and triple infection were common throughout this study. Bbsl double infections occurred 23 times, whereas *B. odocoilei* double infections happened 23 times. A triad of Bbsl, *B. odocoilei*, and *A. phagocytophilum* occurred once as a triple infection.

Table 2: Detection of tick-borne zoonotic pathogens in human-biting *Ixodes* ticks collected in Canada.

Region	Tick species	No. of ticks	Bbsl	B. miy.	B. odo.	B. mic.	Aph
			Questing and Host-acquired				
BC	*I. angustus*	9	0	0	1	0	0
	I. pacificus	16	0	0	8	0	0
	I. scapularis	8	3	0	3	0	0
AB	*I. scap.*	4	2	0	2	0	0
SK	*I. scap.*	5	1	0	1	0	0
MB	*I. scap.*	26	8	0	10	0	1
N. Ont.	*I. scap.*	19	11	1	6	0	0
S. Ont.	*I. scap.*	23	10	0	10	0	2
QC	*I. scap.*	27	11	0	11	0	1
NB	*I. scap.*	28	10	1	8	0	2
NS	*I. scap.*	24	10	0	9	1	1
PE	*I. scap.*	11	3	1	3	0	0
NL	*I. scap.*	5	3	1	2	0	0
			Songbird-infested				
Larva molts to nymph		1	0	0	1	0	0
Nymphs molt to females		3	0	0	2	0	0
Pending molt & damaged		15	2	0	5	0	1
Total		224	74	4	82	1	8

Bbsl, *Borrelia burgdoriferi* sensu lato; B. miy., *Borrelia miyamotoi*; B. odo., *Babesia odocoilei*; B. mic., *Babesia microti*; Aph, *Anaplasma phagocytophylum*. I. scap., *Ixodes scapularis*.

Table 3: Non-human biting *Ixodes* ticks in British Columbia that were not tested for tick-borne pathogens.

Tick species	Life stage	Host(s)
I. auritulus, avian coastal tick	1N, 1F	Brown-headed Cowbird, Golden-crowned Sparrow
*I. rugosus**	1F	Raccoon
I. texanus	3N, 4F	Striped skunk**, 2 raccoons, dog
N, nymph(s); F, females(s)		

*This tick was deposited in Collections at the Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, at the University of Guelph, Guelph, Ontario, Canada.

**This is the first tick-host report of an *I. texanus* tick infesting a striped skunk in Canada, and it was collected 3 May 2024, at New Westminster, BC.

Babesia odocoilei was detected in *I. scapularis* in each province from BC to NL (Table 2). *Babesia odocoilei* was the only pathogen detected in *I. angustus* and *I. pacificus*. Notably, we documented the first-ever *B. odocoilei* in an *I. angustus* tick.

In particular, we present the first record of *I. scapularis* positive for Bbsl in BC. Likewise, we furnish the first documentation of *B. odocoilei* in an *I. scapularis* tick collected in BC.

In this Canada-wide, tick-host-pathogen study, *B. microti* was detected only once, and it was detected in an *I. scapularis* male collected in Nova Scotia.

Incidentally, we collected an *I. angustus* female that had parasitized a Douglas squirrel, *Tamiasciurus hudsonicus*, on 14 August 2023 at Burnaby, BC; this is a novel tick-host record. In addition, we report an *I. angustus* female parasitizing an eastern cottontail, *Sylvilagus floridanus*, at Langley, on 26 July 2024. This infestation is a novel tick-host record.

In BC , we collected *I. angustus*, *I. pacificus* and *I. scapularis* from Lower Mainland, Metro Vancouver, South Vancouver Island Central Vancouver Island, Lower Mainland, and Okanagan Valley.

We detected *Babesia odocoilei* and *Borrelia burgdorferi* sensu lato in all provinces, namely Newfoundland and Labrador, Prince Edward Island, Nova Scotia, New Brunswick, Quebec, Southern Ontario, Northern Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia.

In far-western North America, *I. pacificus* has been the motherlode of tick-borne zoonotic pathogens, especially *B. burgdorferi* s.l. and, now, *B. odocoilei*. Our environmental discoveries in British Columbia show that *I. scapularis* share the limelight with *I. angustus* and *I. pacificus*.

Overall, there were 169 pathogen positives in 224 *Ixodes* ticks. On average the majority of *Ixodes* ticks was infected with a pathogen.

Of special note, an *Ixodes rugosus* female (tick no., 23-5A1A; BIO-23-022) was collected from a raccoon, *Procyon lotor*, on 8 February 2023, at Victoria, BC. The tick species was confirmed by molecular barcoding, and deposited as a voucher specimen in Collections of the Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, at the University of Guelph, Guelph, Ontario.

Molecular detection

Since nested PCR outperformed real-time PCR, we reported nested PCR results. Pathogens are in figure 1 and table 2.

We provide the first report of *B. odocoilei* in *I. angustus* in BC. An *I. angustus* female from a single deer mouse, *Peromyscus maniculatus*, was negative for all 5 pathogens.

Discussion

We provide the first report of *B. odocoilei* in *I. scapularis* in British Columbia, Aberta, Saskatchewan, Manitoba, New Brunswick, Prince Edward Island, and Newfoundland and Labrador. Since *B. burgdorferi*, *Borrelia miyamoti*, *B. microti*, and *A. phagocytophilum* have previously been studied exquisitely, we concentrated on *B. odocoilei*, a sequestering *Babesia*. This novel *Babesia* piroplasmid is capable of infecting

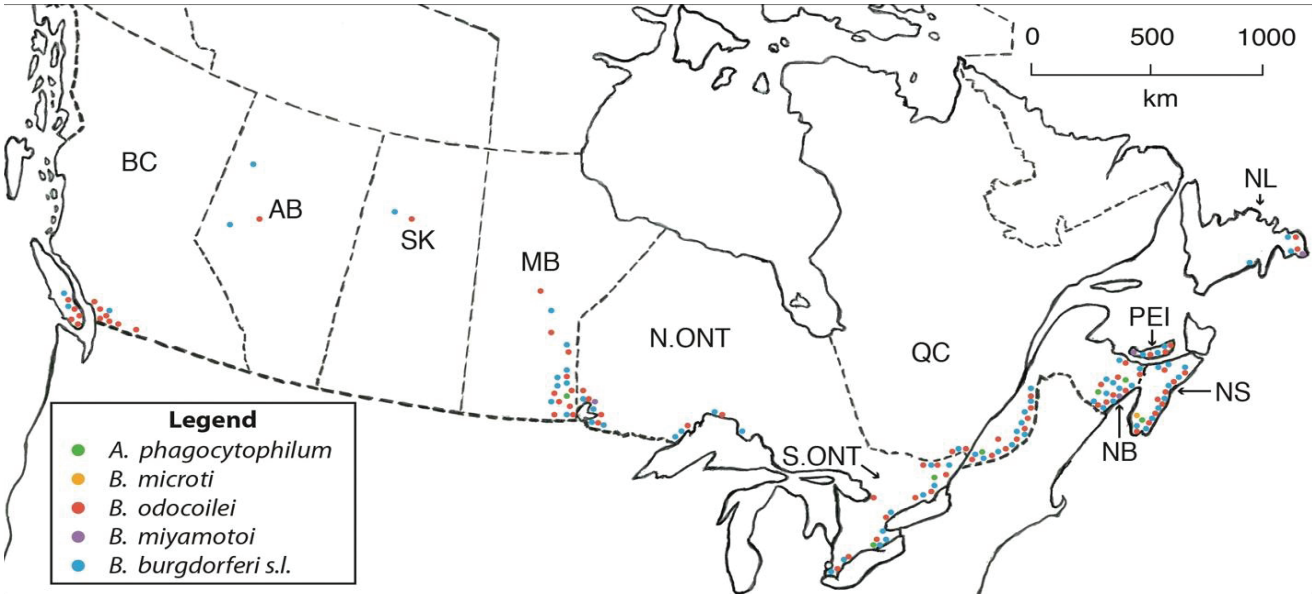

Figure 1 The distribution of five tick-borne zoonotic pathogens in adults of *Ixodes angustus*, *Ixodes pacificus*, and *Ixodes scapularis* across Canada.

humans causing a chronic, insidious, babesial infection. For pathophysiology information on sequestering *Babesia* species, we accessed the veterinary, malaria and *Babesia* literature. Government and licensing colleges must initiate an extensive education program for healthcare practitioners in order to raise the intelligence quotient on tick-borne zoonotic diseases.

Tick identification methods

One technical service group in BC has been using e-photos, taken with smartphones, for tick identification. Unremarkably, smartphones do not have the magnification and resolution to differentiate *Ixodes* species accurately. For instance, an unfed female *I. pacificus*, and an unfed female *I. scapularis* and an unfed female *I. spinipalpis* look identical through the lens of a smartphone. Once a tick becomes partially or fully engorged, it is impossible to identify *Ixodes* ticks using a smartphone. For example, the dorsal surface of unfed females of *I. pacificus*, *I. scapularis*, and *I. spinipalpis* are indistinguishable. In the present study, ticks were identified by a professional acarologist (J.D.S.). In particular, an *Ixodes rugosus* was barcoded (molecular characterization) to confirm the identification.

Dogs as a reservoir of *B. odocoilei*

Based on our results, we suggest that domestic dogs, *Canis familiaris*, are a reservoir of *B. odocoilei*. Significantly, 8 (50%) of the 16 *I. pacificus*, which had parasitized domestic dogs in BC, were infected with *B. odocoilei*. These enzootic occurrences were similar to *I. scapularis*-infested dogs collected in the Huronia region of southern Ontario, which had an infection prevalence of 71% for *B. odocoilei* [33]. Dogs in southwestern BC were also infested with *I. scapularis* adults bearing *B. odocoilei*. Veterinarians and veterinarian technicians should be aware that *B. odocoilei* is frequently present in *Ixodes* ticks that parasitize canine domestic animals.

Capillary and venule blockage

During a *B. odocoilei*-infected *I. scapularis* tick bite, sporozoites enter the blood stream of the host. They promptly enter uninfected Red Blood Cells (uRBCs), and create infected Red Blood Cells (iRBCs). Next, sporozoites develop into trophozoites, and then gradually advance to infective merozoites [66]. As part of the unique pathophysiological reaction, fibrinogen converts to fibrin. Fibrin then bonds with both iRBC and uRBCs and, together, adhere to the endothelium cells (sequestration) [67,68]. Gradually, these fibrin-bonded entanglements occlude capillaries and venules throughout the body. Sequestering *Babesia* spp. (i.e., *B. canis*, *B. odocoilei*) complete their life cycle within these fibrin-bonded entanglements [68]. Therefore, these sequestering *Babesia* remain inaccessible to the spleen and the circulating immune system [68]. Sequestering *Babesia* merozoites obstruct capillaries and venules, and occlude these arterial passageways, especially the brain, which has the smallest capillaries.

Blockage of capillaries by fibrin-bonded entanglements of sequestering *Babesia* compromises mitochondrial function and, therefore, energy production is lessened. Fundamentally, oxygen and nutrients are hindered from reaching the cells. Mitochondria are tiny organelles in cells that generate chemical energy in the form of Adenosine Triphosphate (ATP) [69]. When ATP falters, physical and mental activity is depleted. Thus, fatigue, inflammation, and cognitive dysfunction are key underlying symptoms of human babesiosis caused by *B. odocoilei*.

Babesia odocoilei can complete its life cycle within the fibrin-bonded entanglements throughout the human body especially in the brain which has the smallest capillaries [67,68,70]. In stark contrast, non-sequestering *Babesia* spp. (i.e., *B. microti*) circulate in the arterial system, and are attacked by macrophages, and

also trapped by the spleen (an immunological barrier) [71]. Biogeographically, *B. odocoilei* is widespread in North America and, undisputably, the predominant *Babesia* species continentally. Overseas, *B. odocoilei* was detected in red deer, *Cervus elaphus*, and cattle in the United Kingdom [72] and, likewise, other countries in Europe.

Transovarial transmission and Transstadial passage

Once a gravid female becomes infected with *B. odocoilei*, gametes develop into ookinetes, and then kinetes [73,74]. These kinetes migrate to female ovaries. When mating has occurred, and spring temperatures are favorable, the gravid female lays 1000 kinete-infected eggs and, within 4-6 wk., hatch to infective larvae via transovarial transmission [23,66]. Transovarial transmission is an exquisite, reproductive feature of *I. scapularis* females infected with *B. odocoilei*. These larvae develop in late July and, within a day, start questing for hosts. Thus, *I. scapularis* can transmit *B. odocoilei* to humans. If the infective larvae bite hosts that are not infected with *B. odocoilei*, these larvae still retain *B. odocoilei* throughout the larva-nymph molt and, likewise, the nymph-adult molt (transstadial passage) [23,66]. Once *I. scapularis* larvae become infective they can maintain *B. odocoilei* for generations without feeding on *B. odocoilei*-infected vertebrate hosts. On the contrary, *B. microti* and *B. burgdorferi* are not transmitted transovarially. If, in the event that *I. scapularis* larvae are not infected with *B. odocoilei*, they must acquire a blood meal from a *B. odocoilei*-infected host to molt to *B. odocoilei*-infected nymphs.

Because 1000 larvae from one gravid *I. scapularis* female can be infected with *B. odocoilei* [66], questing larvae produce an alarming public health threat. People visiting or working in parks and forest-dwelling areas render themselves to *B. odocoilei*-infected ticks.

Our findings reveal that people are just as likely to contract human babesiosis as Lyme disease. In fact, our data is concordant with a Lyme disease study in southwestern Ontario with a prevalence of 36% for Bbsl [75]. Since a tiny (0.75 mm) larva is hard to see, its minute size greatly raises the chance of people being bitten, and acquiring *B. odocoilei* infection.

Acute symptomology

Patients with human babesiosis caused by *B. odocoilei* may have a multitude of symptoms. Primary symptoms that are commonly experienced by patients include unyielding fatigue, slow/impaired cognition, anxiety, difficulty remembering, brain fog, memory loss, muscle and joint aches, muscle and joint stiffness, poor balance, clumsiness, disorientation, bladder dysfunction, sleep disturbance, insomnia, nightmares, irritability/rage/aggression, profound/wild dreams, sweats (especially at night), unsteady gait, clumsiness, dizziness, ischemia (slow blood flow), intestinal problems, constipation, chills, heat and cold intolerance, pathogen-induced depression, irritability, air hunger, increased thirst, slow urination, numbness (especially in fingers), long-standing headaches, encephalopathy, progressive dementia, and herxing (Jarisch-Herxheimer reaction) following treatment [5,6]. From a pathophysiology state, hemolysis and thrombocytopenia (reduced number of blood platelets) are regular haematologic manifestations of sequestering *Babesia* [5,6]. Suffice it to say, human babesiosis caused by *B. odocoilei* inflicts major depression in the human population. A comorbidity, such as Lyme disease and human babesiosis caused by *B. odocoilei*, typically exacerbate pain and suffering.

Children with human babesiosis caused by *B. odocoilei* promptly develop mystifying symptoms. Before symptoms set in, they are typically very verbal and high-functioning. As this babesial infection becomes established, they generally have a decline in speech. Anxiety,

insomnia, and behavioral changes typically take hold. As fibrin-bonded entanglements of *B. odocoilei* block capillaries, children often become non-verbal, obtain muscle weakness, and have unsteady balance. As well, bladder and bowel dysfunction are concordant with advanced manifestations.

Chronic symptomology

As the symptoms of human babesiosis caused by *B. odocoilei* advance, symptoms become more pronounced and insidious. These symptoms may include any combination of unending inflammation, absent-mindedness, severe encephalitis, white matter hyperintensities, exertional intolerance, coma, stroke, retinal vasculitis, difficulty walking, shaky, nausea, homicidal tendencies, extreme anger, aggression, emotional (cry for hours), whole body pain, varying moods, severe depression, and suicidal/homicidal ideation. Patients often become bedridden and disabled and, thus, exacerbating and propelling agoraphobia [5,6]. Suicides are familiar, and some patients with chronic human babesiosis caused by *B. odocoilei* result in death [personal communication, Henry Lindner, MD].

Babesia odocoilei merozoites and fibrin strands occlude capillaries and venules, and are recalcitrant to treat. Patients are often labelled with conditions/disorders/maladies including Alzheimer's disease, multiple sclerosis, fibromyalgia, ADHD, oxidative stress, chronic fatigue syndrome, bipolar disorder, psychosomatic depression, irritable bowel syndrome, "long-COVID," long-standing Lyme disease, autoimmune disorder, Susac's syndrome, autism, narcissistic personality disorder and mast cell activation. Healthcare professionals have been sidestepping the actual cause of human babesiosis cause by *B. odocoilei* and, instead, fabricate, falsify, and misdiagnose the actual cause. *Babesia* species are normally transmitted by ticks but may be transmitted by blood transfusion [76,77], organ transplantation [78], and maternal-fetal transmission [79-82]. *Babesia odocoilei* sequesters in the brain, and produces cerebral pathophysiology. Since the brain has the smallest capillaries, occlusions in capillaries and venules deprive cerebral tissue of oxygen and nutrients. As well, cardiovascular disease often occurs because fibrin-bonded entanglements occlude and block capillaries in the heart.

Songbirds disperse pathogen-infected ticks

Neotropical migratory songbirds play a major role in the wide dispersal of songbird-transported ticks [83-88]. Certain passerines (i.e., Common Yellowthroat, Gray-cheeked Thrush) are transported as far north as the boreal forest that spans Canada (Figures 2A,B). Ticks on migratory songbirds are released at stopovers en route along the far-reaching flightpath. Scott, et al. [88] collected *I. scapularis* nymphs as far north and west as northwestern Alberta [84]. After release, blood-fed larvae and nymphs molt, and are ready to conduct host-seeking activities. When a juvenile *I. scapularis* obtains a blood meal, and becomes fully engorged, they undergo transstadial passage (larvae to nymphs &/or nymph to adults). The unfed nymphs and unfed females can bite humans, and transmit tick-borne zoonotic pathogens such as Bbsl, *B. miyamotoi*, *Babesia* spp., and *A. phagocytophilum* [33,48-52].

Tick co-infections

Certain *I. pacificus* and *I. scapularis* ticks in the present study were co-infected with two or more tick-borne zoonotic pathogens. When people are bitten by ticks, these individuals are frequently treated with "one-size-fits-all" antimicrobials. In reality, there are multiple combinations and permutations of tick species and tick pathogens. Polymicrobial infections are seldom taken into account by clinicians. These comorbidities normally require different antimicrobials because of

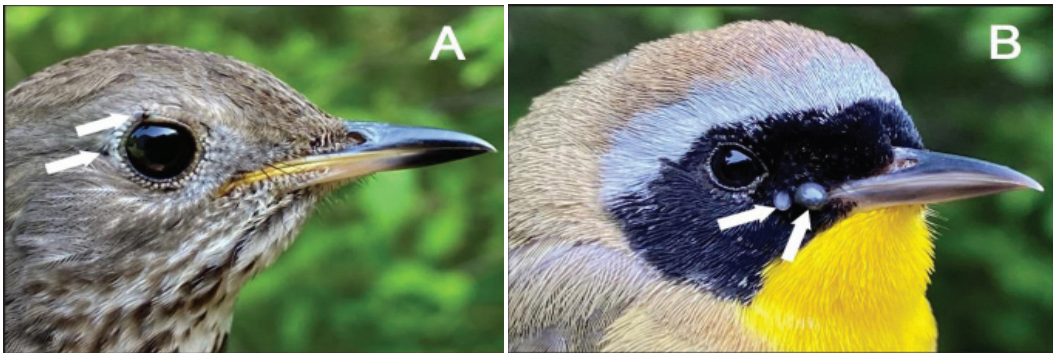

Figure 2 Migratory songbirds infested with ticks: A) Gray-cheeked Thrush parasitized by *I. scapularis* nymphs; B) Common Yellowthroat, male, parasitized by *I. scapularis* nymphs. One nymph molted to a female in 54 d, and one nymph was infected with *Borrelia burgdorferi* sensu lato.

different pathogens. For Bbsl, an antibiotic (i.e., doxycycline, amoxicillin) is commonly administered. For *B. odocoilei*, an antibabesial (i.e., artemisia, primaquine, tafenoquine, itraconazole, ivermectin) is can potentially be ordered. In addition, a fibrinolytic enzyme (i.e., lumbrokinase, serrapeptase, or nattokinase) is efficacious with antibabesial treatment protocol [89]. In chronic patients, antibabesials should be started at a low dose because the Jarisch-Herxheimer reaction can be severe. Healthcare practitioners and pharmacists should never assume that *I. pacificus* or *I. scapularis* are infected with a single pathogen, and a single pill is a cure [90]. When a tick has fed on a person, the tick should be identified by a proficient professional for tick species, and tested for associated tick-borne zoonotic pathogens.

Antimicrobial treatment

Some healthcare practitioners use one doxycycline, 200 mg, as the normal default treatment. Although a single-dose doxycycline for the prevention of Lyme disease may result in the diminution of erythema migrans, most patients failed this single dose regimen, and these study participants proceeded to develop Lyme disease or co-infections. Another shortfall arose when the study only followed the patients for 6 wk [90] and, henceforth, it could not be determined whether the patients went on to develop a long-term, malignant infection, such as chronic Lyme disease [13,14].

Since *B. odocoilei* infections resemble *Plasmodium falciparum* malaria, treatment regimens are often recalcitrant. Because fibrin-bonded entanglements are tightly compacted with merozoites and fibrin, highly compacted capillaries are recalcitrant to treat.

Furthermore, a *B. odocoilei* infection can arise in blood transfusion recipients because donors can be infected. Ruefully, the blood supply in Canada is currently not being screened for *B. odocoilei*, and this shortfall multiplies and tragically, spreads human babesiosis.

Without efficacious, antimicrobial treatment, chronic patients with tick-borne zoonotic diseases, such as *A. phagocytophilum* [8,91], *B. odocoilei*, and Bbsl, can succumb to death [92-94].

Ixodes angustus exhibits vector competence of *Borrelia burgdorferi*

We provide the first-ever *I. angustus* positive for *B. odocoilei*. Historically, Damrow, et al. [95] collected an engorged *I. angustus* female from the eyelid of a 3-year-old girl residing within Washington State in May 1987. She had a bull's-eye rash, and had an IFA titre of 1:256 for Lyme disease. Directly to the north, in BC, researchers detected Lyme disease spirochetes in a wild-caught *I. angustus* [96]. As well, Peavey, et al. [97] found that *I. angustus* is a competent vector

B. burgdorferi sensu stricto, and elucidate vector competence in *I. angustus*. Although we did not detect Bbsl in *I. angustus*, we detected *B. odocoilei* in *I. angustus*–a first-time documentation.

Conclusion

Our findings reveal that *B. odocoilei* plays a prominent role as a human pathogen Canada-wide. Our quintessential results show that when a person is bitten by an *I. scapularis* tick, the person is just as likely to be infected with *B. odocoilei* as the Lyme disease bacterium. In BC, a person is more likely to be bitten by a *B. odocoilei*-positive tick than a Bbsl-positive tick. We unveiled the first epidemiological study showing *I. scapularis* infected with *B. odocoilei* in BC. Biogeographically, we detected *B. odocoilei* in *I. scapularis* ticks for the first time in AB, SK, MB, NB, PE, and NL. Previously, *B. odocoilei* was discovered in N. Ontario, S. Ontario, Quebec, and Nova Scotia, but now, this babesial piroplasmid is documented in all provinces a unique nationwide finding. In the present study, we demonstrate a more pronounced ratio of *B. odocoilei* to *B. microti*, notably 60 to 1. Therefore, *B. odocoilei* is the predominant *Babesia* sp. across Canada. Notably, we provide the first report of *B. odocoilei* in *I. angustus*. In reality, applying a tick prevention acaricide before frequenting a woodland or park adds credence to cutting the risk of contracting tick-borne zoonotic diseases. In order to minimize the incidence of human babesiosis caused by *B. odocoilei*, deer culls are prudent wildlife management. With the occurrence of tick-borne zoonotic pathogens continent wide, the medical profession must become trained in tick-borne zoonotic diseases. Polymicrobial infections must be in the healthcare practitioner's differential. In order to mitigate tick-borne zoonotic pathogens, special professional healthcare attention must be given to test ticks for pathogens. Health care professionals must bolster their continuing medical education training in tick-borne zoonotic diseases.

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Ethical consideration

Ethical approval is not needed to remove ticks from avian and mammalian hosts. Regulatory approval is not required to hold engorged ticks to molt.

Authors' contributions

Conceptualization and design: JDS and CMS. Collection and methodology: JDS. Formal analysis: JDS and CMS. Drafting of manuscript: JDS and CMS. Accuracy of data: JDS and CMS. All authors took part in the this bellwether study, and read and approved the final version of the scientific manuscript.

Competing financial and investments interests

The authors declare that they have no competing financial and investment interests relating to the study.

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