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RESEARCH ARTICLE

Molecular Detection of *Borrelia burgdorferi* sensu lato, *Babesia odocoilei*, *Babesia microti* and *Anaplasma phagocytophilum* in *Ixodes* Ticks in British Columbia, Canada

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Abstract

In total, 76 *Ixodes* ticks were collected in southwestern British Columbia (BC). Five *Ixodes* species (*I. angustus*, *I. cookei*, *I. pacificus*, *I. scapularis*, and *I. spinipalpis*), which bite humans, were collected and tested. Two *Ixodes* spp. (*I. rugosus*, *I. sculptus*), which do not bite humans, were collected and tested. Using real-time and nested PCR, the four pathogens were detected, namely *Borrelia burgdorferi* sensu lato (19/76, 25%), *Babesia odocoilei* (12/76, 16%), *Babesia microti* (1/76, 1%), and *Anaplasma phagocytophylum* (2/76, 3%). We provide the first report of *Ixodes scapularis* parasitizing an eastern cottontail, *Sylvilagus floridanus*, in Canada. We document the first molecular identification of *Ixodes sculptus* parasitizing an American mink, *Neogale vision*; this tick-host parasitism occurred on Vancouver Island, BC. At an established population on mainland BC, *Ixodes pacificus* adults (7/15, 47%) were positive for *Babesia odocoilei*. We report the first *Ixodes pacificus* parasitizing a Mallard Duck, *Anas platyrhynchos*. Based on the findings of this tick-host-pathogen study, healthcare practitioners must be aware that at least 5 different *Ixodes* spp. transmit tick-borne zoonotic pathogens in BC. *Babesia odocoilei* does not respond to doxycycline treatment.

Introduction

Tick-borne zoonotic diseases cause horrific disability worldwide. Once they become chronic, tick-borne diseases are persistent, and recalcitrant to treat. In North America, *Ixodes* ticks can carries more than one pathogen [1]. In fact, four pathogens have been detected in an *Ixodes scapularis* adult [2]. *Ixodes scapularis* has previously been detected in British Columbia (BC) [3]. Another *Ixodes* tick in western Canada is *Ixodes spinipalpis* which is found in parts of Alberta and BC [4]. This nidicolous tick spends much of its time in a darken cavity nestled within a burrow. Researchers have found that *Ixodes spinipalpis* bites humans, and can transmit

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the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (hereafter Bbsl) [5-10]. Bbsl was first discovered in *Ixodes scapularis* ticks collected on Long Island, NY [11]. Around the globe, there are at least 24 valid genospecies of Bbsl.

Acarologists have found that *Babesia odocoilei* (Apicomplexa: Piroplasmidae: Babesiidae), a single-celled, red blood cell parasite is equally as prevalent as Bbsl across Canada [12]. Also, these researchers discovered that the ratio of *Babesia odocoilei* to *Babesia microti* in *Ixodes scapularis* adults was 60 to 1 nation-wide [12]. Markedly, *Babesia odocoilei* was first discovered in patients in Ontario, Canada [13,14]. The black-tailed deer, *Odocoilei hemionus columbianus*, are prominent cervids in southwestern BC, and are reservoirs of *Babesia odocoilei* [15]. In addition, this intracellular piroplasmid has been documented in the U.K. [16] and in the E.U. [17]. This piroplasmid is normally transmitted by a tick bite [18], but may be transmitted by blood transfusion [19], organ transplantation [20], and maternal-fetal transmission [21-24]. Whenever a pregnant person has been bitten by a tick, obstetricians must include neonatal *Babesia* in the differential diagnosis when screening for neonatal sepsis [22].

Ixodes cookei, the groundhog tick, is common in eastern Canada [4], but was previously found in BC [25]. This tick species does not parasitize birds [4].

Ixodes augustus was documented in *Ixodes* ticks in BC [12,25]. As well, this ixodid tick does not parasitize birds [4].

The primary objective of this study was to screen ticks for Bbsl, *Babesia odocoilei*, *Babesia microti*, *Bartonella* spp., and *Anaplasma phagocytophilum*, and embark on finding the prevalence of these tick-borne zoonotic pathogens in southwestern BC.

Materials and Methods

Tick collection

Veterinarians, veterinary technicians, veterinary associates, wildlife rehabilitators, and the public collected ticks from domestic and wildlife mammals. Flagging low-level vegetation complimented collection. At the beginning of the study, 2 mL micro tubes, each containing 95% ethyl alcohol, were sent to veterinary participants. Vials for live ticks were sent to wildlife rehabilitators. At the end of the collection period, ticks were returned to the laboratory (J.D.S.) for identification using taxonomic tick keys [4,26-28]. Three *Ixodes* ticks were taken to the Centre for Biodiversity Genomics, University of Guelph for molecular identification.

We document the first account of *Ixodes spinipalpis* (nidicolous tick) molting from nymphs to adults (transstadial passage). Fully engorged *Ixodes spinipalpis* nymphs were held in darkness to molt to adults. The temperature was set at 20-25°C, and the humidity at 80-95%. Technical assistance was obtained from a scientist in Belgium—see Acknowledgments.

Molecular analysis

DNA extractions and PCRs were completed by Geneticks Inc. Ticks were bisected lengthwise. Each half was homogenized by beating a 400 µl DNA/RNA shield (ZymoResearch) with a mix of 2.3 mm and 0.1 mm Zirconia/Silica beads (BioSpec Products). Samples were subjected to two subsequent runs for 5 min at 2400 RPM in a Mini-Beadbeater-96 (BioSpec Products). Total nucleic acid was isolated from homogenized tick halves using the Quick-DNA/RNA Pathogen Miniprep (Zymo Research) following the manufacturer's instructions.

A combination of real-time PCR and nested PCR assays were used for pathogen detection. The primers and probes used in this study are listed in Table 1 below. All samples were tested for

Table 1. Primers and probe used for pathogen detection.

Genus/Species	Gene	PCR Type	Primer Name	Sequence (5'-3')	Amplicon Size	Reference
Borrelia spp.	23s IGS	qPCR	Bb23Sf	cgagtcttaaaagggcgatttagt	75	[29]
			Bb23Sr	gcttcagcctggccataaatag		
			Bb23SProbe	FAM-agatgtggtagacccgaagccgagtg-ECLIPSE		
Borrelia miyamotol	flaB	qPCR	flaBf	ccttcaagtactccagatccattg	102	[30]
			flaBr	aacaaagacggcaagtacgatc		
			ospAprobe	FAM-TGCAACAGTAGACAAGCTTGAGCT-ECLIPSE		
Anaplasma phagocytophilum	msp2	Nested PCR	AnaP44OutL1-F	GTAGAAGAAACCGCCCTAAT	850	[31]
			AnaP44OutL1-R	TCTATGTTGGTTTGGATTACAG		
			MSP3F	CCAGCGTTTAGCAAGATAAGAG	334	[32]
			MSP3R	GCCCAGTAACAACATCATAAGC		
Babesia microti	18s rRNA	Nested PCR	Bab1	CTTAGTATAAGCTTTATACAGC	238	[33]
			Bab4	ATAGGTCAGAAACTTGAATGATACA		
			Bab2	GTTATAGTTTATTTGATGTTC	155	
			Bab3	AAGCCATGCGATTCGCTAAT		
Babesia odocoitel	18s rRNA	Nested PCR	Bab306R_RCF	TTTCTGCGTCACCGTATT	331	[34]
			BabGeninR2	ACGACGGTATCTGATCGTCT	311	
			odo563	CCGTATTTGACTTTGTCGACTGT	311	[31]
			BabGeninR1	TCTGATCGTCTTCGATCCC		
Bartonella spp.	RibC	Nested PCR	RibC-1F	CGGATATCGGTTGTGTTGAA	309	[35]
			RibC-1R	CATCAATRTGACCAGAAACCA		
			RibC-2F	GCATCAATTGCTTGTTCA	182	
			RibC-2R	CCCATTTCATCACCCAAT		

the presence of *Borrelia* spp., *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*, and *Bartonella* spp. All *Borrelia* testing was performed using real-time PCR in 30 µl reaction volumes using 15 µl of PC RBIO Probe Blue Mix (PCRBiosystems). Subsequently, 800 nM of both forward and reverse primers, 250 nM of probe, and 10 µl of extracted total nucleic was used as the template. Reactions were subjected to an initial denaturation of 8 min at 95˚C followed by 40 cycles at 95C for 10 sec, and 60˚C for 30 sec. Real-time PCR reactions were performed using a Stratagene Mx3005P qPCR machine (Agilent Technologies). To interpret qPCR results, the following algorithm was used: samples that tested positive for both *Borrelia* spp., *B. miyamotoi* were considered positive for *B. miyamotoi*. Samples testing positive for *Borrelia* spp., but negative for *B. miyamotoi*, were considered positive for Bbsl. Samples that tested negative for both *Borrelia* spp. and *B. miyamotoi* were considered negative for all *Borrelia* spp.

Detection of *A. phagocytophilum*, *B. microti*, *B. odocoilei*, and *Bartonella* spp. was performed by nested PCR in 25 µl reaction volumes using 12.5 µl of 2x Taq FroggaMix (Frogga Bio Scientific Solutions). Next, 400 nM of both forward and revere primers, and 2 µl of template. The outer reaction conditions for *A. phagocytophilum* included an initial denaturation of 95˚C for 10 min followed by 35 cycles of 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

which was performed at 55˚C, and 40 total reaction cycles were used. The outer reaction conditions for *Babesia odocoilei* included an initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 30 sec, then 58˚C for 30 sec, 72˚C for 30 sec, and a single final extension of 72˚C for 10 min. The inner reaction state was identical, except annealing was performed at 63˚C for 15 sec, and extension was performed at 72˚C for 20 sec. Both outer and inner reaction conditions for *Babesia microti* included an initial denaturation at 95˚C for 10 min, followed by 35 cycles of 95˚C for 30 sec, 55˚C for 30 sec, 72˚C for 30 sec, and a single final extension of 72˚C for 10 min. For *Bartonella* spp., the outer reaction included an initial denaturation at 95˚C for 10 min, followed by 40 cycles at 95˚C for 30 sec, 57˚C for 30 sec, 72˚C for 60 sec, and a single final extension of 72˚C for 10 min. The inner reaction was identical, except the annealing temperature was 52˚C, and the extension time was reduced to 30 sec. All nested PCR reactions were performed in a MJ Research PTC-225 Tetrad Thermocycler (BioRad).

Results

Overall, twelve established populations of *Ixodes* ticks (i.e., *I. pacificus*, *n* = 8; *I. scapularis*, *n* = 2; and *I. spinipalpis*, *n* = 2) were detected within the following regions: Metro Vancouver, Fraser Valley, Okanagan Valley, Sea to Sky Corridor, Whistler Valley, Greater Victoria, Cowichan Valley, and Sunshine Coast. Other *Ixodes ticks* collected included *I. angustus*, *I. cookei*, *I. rugosus*, *I. sculptus,* and *I. texanus*. The *I. sculptus* was collected from an American mink, *Neogale vision*, on 10 March 2025 at Saanich, BC. This parasitism is the first report of an *I. sculptus* on Vancouver Island, BC.

Eight *I. spinipalpis* ticks were collected from an eastern cottontail on 10 April 2025 at Victoria, BC. Five of 8 (63%) *I. spinipalpis* were positive for Bbsl. One of these gravid females laid viable eggs that hatched to active larvae (Figure 1).

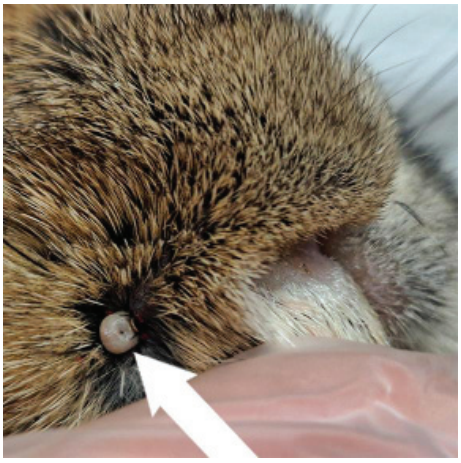

Figure 1 Eastern cottontail parasitized by a partially engorged *Ixodes spinipalpis* female. Credits: Christina Carrieres.

We collected an *Ixodes rugosus* female from a striped skunk, *Mephitis mephitis*, on 31 March 2025 at West Vancouver, and this female was positive for *Babesia microti*. This tick-host-pathogen record is a first.

We collected an *Ixodes spinipalpis* male from an eastern cottontail at Sidney, BC on 15 May 2025; this male was positive for *Anaplasma phagocytophilum*—a tick-host-pathogen first.

Two *I. scapularis* females were collected from an eastern cottontail on 20 May 2025 at North Saanich, BC. This tick-host finding confirms that this tick species is indigenous in BC.

We flagged a site in the Hope area (Fraser Valley), and 7 of 15 (47%) of *Ixodes pacificus* adults were positive for *Babesia odocoilei*. This is the premiere documentation of *B. odocoilei* in an establish population in Canada. Paradoxically, all *Ixodes pacificus* collected and tested from this site were void of Bbsl.

An *I. scapularis* female collected on 26 May 2025 from a dog in the Whistler Valley was infected with *Babesia odocoilei*. Likewise, a partially engorged *I. pacificus* female was collected on 10 March 2025 at Duncan, BC, and was positive for *Babesia odocoilei.*

A slightly engorged *I. pacificus* female was collected from a dog residing at Squamish, BC (Sea to Sky Corridor), and it was positive for *Babesia odocoilei*.

Two *I. pacificus* nymphs were collected from a Mallard Duck on 13 May 2025 at Victoria, BC. These engorged fully engorged nymphs molted to females in 40 d and 41 d. We provide the first documentation of *Ixodes pacificus* parasitizing a Mallard Duck.

Three ticks were sent to the Centre for Biodiversity Genomics for molecular identification, and included: *Ixodes cookei*, 24-5A107; *Ixodes sculptus*, 25-5A6; and *Ixodes spinipalpis*, 24-5A106B1.

We did not find *Bartonella* spp. in any of the ticks tested during the present study.

One of the unique features of *I. spinipalpis* females is retrograde auriculae; quite often they are broken off during tick removal. We molted fully engorged nymphs to adults to confirm their morphological identification. The presence of retrograde auriculae in females is a distinguishing physical characteristic of *I. spinipalpis*.

Four pools of viable ticks (10/pool) were tested for transovarial transmission; all were negative.

Ixodes ticks (i.e., *I. pacificus*, *I. scapularis*) infesting cats and dogs were positive in various locations for Bbsl and *Babesia odocoilei*. For example, *Ixodes pacificus* were positive for *Babesia odocoilei* at the following locations: Hope, Sooke, Squamish, Victoria), and *Ixodes scapularis* were positive for *Babesia odocoilei* at Whistler (Whistler Valley). *Ixodes* ticks: *Ixodes pacificus* and *Ixodes scapularis*) were positive for Bbsl at Duncan, Nanaimo, North Saanich, Saanich, Victoria, Vancouver, Whistler, and Okanagan.

Discussion

During this tick-pathogen-host study, we collected five different *Ixodes* species in southwestern BC that are vectors of the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato complex. In order to obtain a better understanding of the pathophysiology of sequestering *Babesia* species, we gleaned pertinent information from the veterinary and *Plasmodium falciparum* malaria literature. *Babesia odocoilei*, a single-celled red blood celled parasite, has fibrin-bonding entanglements that occlude capillaries and venules. Since human babesiosis caused by *Babesia odocoilei* is an energy-draining disease, it has taken the spotlight continent wide. Human babesiosis is a nationally notifiable disease in Canada. However, valid cases are not being reported. The lack of due diligence by Public Health Agency of Canada in reporting cases is counterintuitive. Clinicians must recognize that human babesiosis caused by *Babesia odocoilei* is serious, and requires a different antibabesial regimen for treatment than Bbsl.

Ticks on acaricide-treated dogs

When *Ixodes* ticks are removed from dogs, there is every likelihood that the canine has been treated with an acaricide (i.e., fluralaner, lotilaner). Since ticks on these treated dogs are generally dead, testing them for tick-borne zoonotic diseases is worthless. Therefore, the degradation of the DNA in these dead ticks greatly reduces the prevalence of tick-borne zoonotic pathogens. As a result, our prevalence results are reduced, but we were not able to determine the extent of the reduction.

Ixodes scapularis indigenous in BC

We found that *Ixodes scapularis* is an ectoparasite of eastern cottontails in BC. The collection of *Ixodes scapularis* females from eastern cottontails confirms that this tick species is established in BC. *Ixodes scapularis* were previously collected from a Mallard duckling on Vancouver Island, BC [3]. Now, we collected *Ixodes scapularis* from eastern cottontails, and these lagomorphs had no out-of-province

travel. The infestation of *Ixodes scapularis* on eastern cottontails confirmed that they were locally-acquired and, therefore, are indigenous in BC. Unquestionably, *Ixodes scapularis* is established in the Pacific Northwest.

Historically, Banerjee SN, et al. [36,37] documented Bbsl in 24 established populations of *Ixodes pacificus* in southwestern BC. In the present 6-mo study, we documented 12 established populations consisting of three tick species, namely *I. pacificus*, *I. scapularis*, and *I. spinipalpis*.

Birds disperse *Ixodes* ticks

Birds play a dynamic role in the wide dispersal of certain songbird-transported, *Ixodes* ticks in BC. They include raptors (Order: Falconiformes) [38], gallinaceous birds (Order: Galliformes) [39], dabbling ducks (Order: Anseriformes) [3], and passerines (Order: Passeriformes) [40] (Figure 2). Whenever fully engorged larvae and nymphs drop from these avian hosts, they must go through a molt (transstadial passage) for 5 to 8 wk before they are ready to take the next blood meal from the next suitable host, including a pet or a human. Markedly, scientists have detected pathogens (i.e., Bbsl, *Babesia odocoilei*, *Anaplasma phagocytophilum*) in the brachial blood of songbirds during the nesting period [41]. Notably, *Ixodes scapularis* larvae and nymphs can transmit tick-borne zoonotic pathogens, such as Bbsl [11] and *Babesia odocoilei* [13,14] during a blood meal. Whenever, anyone frequents a wooded area in temperate weather, they heighten their risk of being bitten, and becoming infected with a tick-borne zoonotic pathogen. Such co-infections make diagnosis and treatment more complex because they require entirely different antimicrobials. Notably, passerine migrants play an inherent function in the wide dispersal of songbird-transport ticks across the Canadian landscape [12,25,42-49]. Comorbidity confounds treatment, especially when a sequestering *Babesia* is concerned.

Discovery of established populations of *Ixodes* species

Three species of *Ixodes* ticks (i.e., *I. pacificus*, *I. scapularis*, *I. spinipalpis*) formed established populations. These populations were found on both Vancouver Island and the mainland. The standard criteria (6 of one life stage, or then, 2 or more life stages) for an established population that pinpointed 12 locations [50,51]. Depending on the individual population, ticks were collected opportunistically from domestic and wildlife mammals, a Mallard and, also, by flagging in southwestern BC. The term "deer tick" is a misnomer because *Ixodes scapularis* has at least 150 vertebrate (avian, mammalian, reptilian) hosts [27].

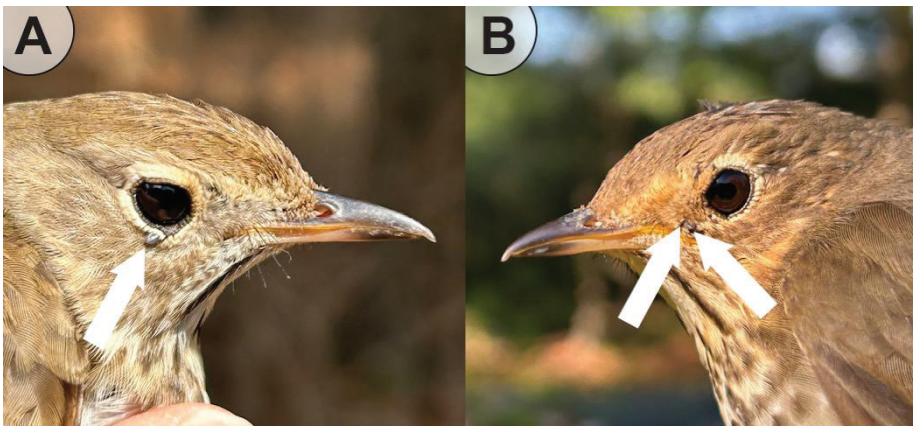

Figure 2 A). Hermit Thrush parasitized by an *I. scapularis* nymph. B). Common Yellowthroat infested by *I. scapularis* nymphs. Both have breeding grounds in BC. Credits: Nancy Furber.

Eastern cottontails are reservoirs of *Borrelia burgdorferi* sensu lato

Eastern cottontails in southwestern BC were originally from Missouri [52]. Researchers previously detected Bbsl in eastern cottontails in southeastern Missouri [52]. When we collected *Ixodes spinipalpis* from these lagomorphs, we found the infection prevalence was surprising high suggesting these rabbits are a reservoir of Bbsl. Coyotes, *Canis latrans*, are a predator of eastern cottontails, and will help to keep the lagomorph population in check.

Sequestering *Babesia* is recalcitrant

When an *Ixodes scapularis* tick is infected with *Babesia odocoilei*, it stores kinetes in its salivary glands. At tick bite, these kinetes are converted to infective sporozoites to adapt to the new host. In the blood stream, sporozoites gradually change to infective trophozoites, and then to infective merozoites [53]. When a *Babesia*-vector tick (i.e., *I. pacificus*, *I. scapularis*) feeds, the kinetes change into infective sporozoites. A new infection, called human babesiosis caused by *Babesia odocoilei* is revealed [13,14]. Once the infection is established, fibrinogen converts to fibrin, and adheres to the endothelium walls (cytoadherence) [54]. At the same time, fibrin combines with infected red blood cells (iRBCs) and uninfected red blood cells (uRBCs). Fibrin-bonded entanglements occlude capillaries and venules, and these occlusions (iRBCs, uRBCs, fibrin) block the flow of blood (sequestration}, especially in the smallest capillaries in the brain followed closely in diameter as gut capillaries [55]. Many capillaries and venules throughout the body become partially or completely occluded. Sequestering *Babesia* spp. (i.e., *B. canis*, *B. odocoilei*) can complete their life cycle within these fibrin-bonded entanglements and, therefore, remain isolated from the circulating immune system and spleen [56]. In essence, *Babesia odocoilei* can survive and propagate in these self-sustaining microcosms, in perpetuity. Sequestering *Babesia* spp. can be extremely recalcitrant to treat, and patients may have a fatal outcome [57,58].

Babesia odocoilei survival strategy

Babesia odocoilei has several attributes to survive in suitable hosts. First, this piroplasmid is pleomorphic (diverse forms) in the human body. These forms include sporozoites, trophozoites, merozoites and gametocytes. Second, this intraerythrocytic piroplasmid forms fibrin-bonded entanglements for sequestration in capillaries and postcapillary venules. *Babesia odocoilei* sequesters in the brain causing cerebral pathophysiology, namely encephalitis. This babesial piroplasmid constrains the function of mitochondria to produce ATP efficiently and, thus, this zoonosis becomes an energy-zapping disease [59]. When a co-infection of Bbsl and *Babesia odocoilei* is present, *Babesia odocoilei* stands in the way of Lyme disease recovery. Of medical importance, doxycycline does not treat *Babesia* infections [60]. The symptoms of human babesiosis caused by *Babesia odocoilei* are forthcoming in Table 2.

In some cases, patients of human babesiosis caused by *Babesia odocoilei* have fatal outcomes (Dan Cameron, MD). Since *Babesia odocoilei* is a sequestering *Babesia* sp., fibrin-bonded entanglements occlude capillaries and post-capillary venules, and cause unrelenting fatigue and severe disability. This tick-borne zoonosis is recalcitrant to treat [13,14]. In contrast, *B. microti* is a non-sequestering *Babesia* species, and has the propensity to be relatively easy to treat. Many people contend that they have dementia, but in reality, they may have a tick-borne zoonotic disease, namely human babesiosis caused by *Babesia odocoilei* [13,14].

Labeling by clinicians

When healthcare practitioners have no explanation for mental, emotional, and physical dysfunctions, they label febrile patients with

Table 2. Symptoms of human babesiosis caused by *Babesia odocoilei*.

Symptoms can include any combination of the following:		
Early-onset symptoms		
unrelenting fatigue	ongoing inflammation	cognitive impairment
legs ache/restless legs	dysphoric (intense unease)	unexplained pain, sore eyes
brain fog/dyslexia	decreased blood pressure	panic attack, feel scared
delirium/disorientation	hallucinations/head pressure	abnormal/wild dreams
anxiety/cry easily, moody	difficulty remembering	dementia, memory loss
dizziness, blurred vision	liver ache from toxin/infection	muscle aches/joint pain
vascular occlusions	numbness, numb hands	irritability/rage/aggression
poor balance/clumsiness	unsteady gait/difficult walking	sore head/pressure in head
bladder dysfunction	lethargic bowels/constipation	fluctuation of emotions
periods of being in a daze	air hunger, shortness of breath	sleep disturbances/insomnia
numbness in fingers/face	chills/heat and cold intolerance	sweats (especially at night)
increased thirst	lack of reading comprehension	nausea/abdominal pain
nerves on fire/in a daze	pathogen-induced depression	loss of interest in hobbies
Late-onset symptoms		
Late-onset symptoms typically become chronic at 6 months.		
chronic headaches	anhedonia (inability to feel joy)	dysautonomia nervousness
thyroid-like signs	feel sorrow/misery/emotional	peripheral neuropathy
enhanced dementia	reading comprehension impaired	ischemia (slow blood flow)
seizures/coma/stroke	white matter hyperintensities	suicidal/homicidal ideation
lack of balance/shakes	motion sickness/difficulty walking	leg weakness/muscle spasm
gruelling fatigue stretches chronic encephalopathy	relentless disability intolerance to do physical activity	dyslexia (trouble read/write) severe hemolysis

familiar morbidities, such as chronic fatigue syndrome, fibromyalgia, psychotic depression, Rasmussen's syndrome, ADHD, mast-cell activation syndrome, multiple sclerosis, dementia, POTS, Tourette's syndrome, unexplained autoimmune issues, and more. Quite often these illnesses are a tick-borne zoonotic disease, such as Lyme disease or human babesiosis.

Anyone with a known or suspected tick bite regularly assumes that the pathogen is the Lyme disease bacterium. However, scientists have found that *Ixodes* ticks are just as apt to be infected with *Babesia odocoilei* as *Borrelia burgdorferi* sensu lato [12]. This default strategy, and subsequent treatment regimen for Lyme disease, fails to work if a sequestering *Babesia* sp. is present. Of medical importance, doxycycline does not treat *Babesia* infections [60]. Also, when treatment for *Babesia odocoilei* is halted, symptoms can recrudesce [13,61]. One inescapable fact remains: sequestering *Babesia* is hard to treat effectively with standard antibabesials [13,61].

Conclusion

Southwestern BC has at least five *Ixodes* species that bite humans. We found that *Ixodes pacificus*, *Ixodes scapularis*, and *Ixodes spinipalpis* harbor *Babesia odocoilei*, and these *Ixodes* ticks can transmit this pathogen to humans. We also discovered the first established population of *Ixodes pacificus* positive for *Babesia odocoilei*. We provide the first report of *Ixodes pacificus* (nymphs) parasitizing a Mallard Duck. We provide the first account of *Ixodes scapularis* parasitizing an eastern cottontail in Canada.

The presence of several *Babesia odocoilei*-infected *Ixodes spinipalpis* parasitizing a single eastern cottontail indicates this host is a reservoir. Based on our collection of *Ixodes* ticks, namely *Ixodes pacificus*, *Ixodes scapularis*, and *Ixodes spinipalpis*, we exposed 12 established populations in southwestern BC. With the overpopulation of deer, predators (i.e., coyotes) are needed to manage deer numbers and, moreover, the government game management programmes must be channeled to vigorously cull the deer herds to mitigate *Babesia odocoilei*. Emphatically, clinicians must realize that patients who are bitten by *Ixodes* ticks are just as likely to be infected with *Babesia odocoilei* as *Borrelia burgdorferi* sensu lato. Explicitly, doxycycline does not treat human babesiosis caused by *Babesia odocoilei*.

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Ethical consideration

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

Authors' contribution

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. Both authors read and approved the final version of this scientific manuscript.

Competing financial and investment interests

The authors declare that they have no competing financial or investment interests relating to this study.

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