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RESEARCH ARTICLE

# Zoonotic Pathogens *Anaplasma phagocytophilum*, *Babesia microti*, *Babesia odocoilei*, and *Borrelia burgdorferi* Sensu Lato Detected in *Ixodes scapularis* Ticks Collected at an Established Population in Eastern Canada

**John D Scott* and Catherine M Scott**

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

## Abstract

Tick-borne zoonotic diseases baffle clinicians and traumatize patients worldwide. We provide the first documentation of four different tick-borne zoonotic pathogens in an established population of blacklegged ticks, *Ixodes scapularis*, located in eastern Canada. Using real-time and nested PCR we detected 4 pathogens in *I. scapularis* adults as follows: *Borrelia burgdorferi* sensu lato (s.l.), 17/25 (68%); *Babesia odocoilei*, 10/25 (40%); *Babesia microti*, 2/25 (8%); and *Anaplasma phagocytophilum*, 3/25 (12%). In addition, we found *B. burgdorferi* s.l. and *B. odocoilei* juxtaposed in *I. scapularis* adults. Moreover, polymicrobial pathogens can be condensed in a single tick bite. Symptoms of human babesiosis caused by *B. odocoilei* are listed. *Babesia odocoilei* is a sequestering *Babesia* sp. that is recalcitrant to treat. Clinicians must be aware that this intraerythrocytic parasite is medically different to treat than the Lyme disease bacterium. Both of these tick-borne zoonotic diseases can be persistent, and often chronic. In reality, there is no such condition as "Post-Treatment Lyme Disease Syndrome (PTLDS)."

## Introduction

Tick-borne zoonotic diseases induce untold veterinary, medical, and economic woes globally. Additionally, they cause profound family discord. The blacklegged tick, *Ixodes scapularis* (Acari: Ixodidae), is the primary vector of at least seven tick-borne zoonotic pathogens. They include the genospecies of the Lyme disease bacterium, *Borrelia burgdorferi* sensu lato (s.l.) complex

***Corresponding author(s): John D Scott,** Upper Grand Tick Research Centre, 365 St. David Street South, Fergus, Ontario, Canada N1M 2L7, **Email:** jkscott@bserv.com; **DOI:** 10.37871/jbres2233

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[1], *Babesia odocoilei* [2,3], *Babesia microti* [4], *Anaplasma phagocytophilum* [5], *Borrelia miyamotoi* [6], *Ehrlichia muris eauclairensis* [7], and the virus of Powassan Virus Disease [8]. Of these human pathogens, *B. odocoilei* is the most recently discovered tick-borne zoonotic pathogen in humans [2,3].

By taxonomy, *Babesia odocoilei* (Apicomplexa: Piroplasnidae: Babesiidae) is an intracellular, red blood cell piroplasmid which is pathogenic to humans [2,3]. This intraerythrocytic microbe has wide distribution across North America [9], the United Kingdom [10], and European Union [11]. *Babesia odocoilei* is a sequestering *Babesia* sp. that is a virulent cousin of *Plasmodium falciparum*, a causative microorganism of malaria.

The main reservoirs of *B. odocoilei* are cervids (e.g., white-tailed deer, *Odocoileus virginianus*) [12,13] and, also, bighorn sheep, *Ovis canadensis nelsoni* [14]. Ornithologically, *B. odocoilei* has been detected in songbird-transported *I. scapularis* larvae and nymphs [9,15-21]. As well, *B. odocoilei* has been detected in brachial blood of songbirds [22]. Songbirds (Order: Passeriformes; Suborder: Passeri), especially neotropical songbirds, play a primary role in the wide dispersal of songbird-transported ticks. When *I. scapularis* larvae and nymphs molt to the next live stage, they can transmit *B. odocoilei* to humans, and initiate human babesiosis caused by *B. odocoilei* [2,3]. Since *B. odocoilei* is endogenous (living within a host) in various ground-frequenting songbirds, birds help to propagate the enzootic cycle of *B. odocoilei*, and other tick-borne zoonotic pathogens. Notably, during a Canada-wide, tick-host-pathogens study, acarologists found that the natural ratio of *B. odocoilei* to *B. microti* in *I. scapularis* adults to be 60:1 [9].

The prevalence of *B. burgdorferi* s.l. and *B. odocoilei* are closely balanced in *I. scapularis* ticks across North America. Scientists have found that the prevalence of these two pathogens (i.e., *B. burgdorferi* s.l., *B. odocoilei*) in *I. scapularis* nationwide to be 40% and 36%, respectively [9].

*Babesia odocoilei* evades and suppresses the host's immune system, and produces life-long infections in immunocompetent hosts, including humans. Dementia, a common symptom of human babesiosis caused by *B. odocoilei*, is escalating at an exponential rate in Canada. Clinicians are either side-stepping this piroplasmid, or they are labeling it as dementia. Deeply troubling, patients often have nowhere to go for knowledgeable healthcare. From a medical standpoint, *B. odocoilei* requires a totally different antibabesial treatment regimen than other bacterial infections [9].

The primary aim of this tick-pathogen study was to determine the prevalence of four different tick-borne zoonotic pathogens in an established population of *I. scapularis* adults in Canada.

## Material and Methods

### Tick collection

*Ixodes scapularis* adults were collected by flagging a five-ha site in Lanark County which is located within the Canadian Shield in eastern Ontario. Red oak, *Quercus rubra*; white oak, *Quercus alba*; white ash, *Fraxinus americana*; and eastern white pine, *Pinus strobus*, were the primary tree species. Flagging focused along the edge of an arboreal area. This ecotone zone consisted of tree seedlings, sumac seedlings, serviceberry, ferns, and invasive Amur honeysuckle, *Lonicera maackii*. These berries are eaten by small mammals that act as suitable hosts.

Since acorns are high in carbohydrates and fats, white-tailed deer eat them to replenish their energy reserves. They are a favorite food in the late fall to help build up fat reserves for the leaner winter months. Deer are reservoirs of *B. odocoilei*, but on the flip side, deer are not reservoirs of *B. burgdorferi* s.l.

*Ixodes scapularis* adults were collected by flagging low-level, dry vegetation in late April 2025. Adults were put in a ziplock plastic bag, and later put in 2 mL micro tubes containing 95% ethyl alcohol. Ticks were sent to the laboratory (J.D.S.) to be identified. An Olympus stereoscopic microscope (SZX16) and authoritative taxonomic keys were used for confirmation of identification [23,24].

## Molecular analysis

All DNA extractions and PCRs were completed by Geneticks Inc. The primers and probes used in this study are listed in Table 1 below.

Adult ticks were bisected longitudinally. 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. All samples were tested for the presence of *Borrelia*

**Table 1:** Primers and probes used to detect pathogens harbored by *Ixodes scapularis* ticks.

| Genus/Species | Gene | PCR Type | Primer Name | Sequence(5'-3') | Amplicon Size | Reference |
|---|---|---|---|---|---|---|
| *Borrelia* spp. | 23s IGS | qPCR | Bb23Sf | cgagtcttaaaagggcgatttagt | 75 | [25] |
| | | | Bb23Sr | gcttcagcctggccataaatag | | |
| | | | Bb23SProbe | FAM-AGATGTGGTAGAC-CCGAAGCCGAGTG-ECLIPSE | | |
| *Borrelia miya-motoi* | flaB | qPCR | flaBf | CCTTCAAGTACTCCAGATCCATTG | 102 | [26] |
| | | | flaBr | AACAAAGACGGCAAGTACGATC | | |
| | | | flabProbe | FAM-TGCAACAGTAGACAAGCTT-GAGCT-ECLIPSE | | |
| *Anaplasma phagocy-tophilum* | msp2 | Nested PCR | AnaP44OutL1-F | GTAGAAGAAACCGCCCTAAT | 850 | [27] |
| | | | AnaP44OutL1-R | TCTATGTTGGTTTGGATTACAG | | |
| | | | MSP3F | CCAGCGTTTAGCAAGATAAGAG | 334 | [28] |
| | | | MSP3R | GCCCAGTAACAACATCATAAGC | | |
| *Bsbesia microti* | 18s rRNA | Nested PCR | Bab1 | CTTAGTATAAGCTTTTATACAGC | 238 | [29] |
| | | | Bab4 | ATAGGTCAGAAACTTGAATGA-TACA | | |
| | | | Bab2 | GTTATAGTTTATTTGATGTTC | 155 | |
| | | | Bab3 | AAGCCATGCGATTCGCTAAT | | |
| *Babesia odo-coilei* | 18s rRNA | Nested PCR | Bab306R_RCF | TTTCTGCGTCACCGTATT | 331 | [30] |
| | | | BabGenlnR2 | ACGACGGTATCTGATCGTCT | 311 | [27] |
| | | | odo563 | CCGTATTTTGACTTTTGTCGACTGT | 311 | |
| | | | BabGeninR1 | TCTGATCGTCTTCGATCCCC | | |
| *Bartonella* spp. | RibC | Nested PCR | RibC-1F | CGGATATCGGTTGTGTTGAA | 309 | [31] |
| | | | RibC-1R | CATCAATRTGACCAGAAACCA | | |
| | | | RibC-2F | GCATCAATTGCTTGTTCA | 185 | |
| | | | RibC-2R | CCCATTTCATCACCCAAT | | |

spp., *Borrelia miyamotoi*, *A. phagocytophilum*, *B. microti*, *B. 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 95°C for 10 sec, and 60°C for 30 sec. Real-time PCR reactions were performed using a Stratagene Mx3005P qPCR machine (Agilent Technologies).

Samples testing positive for *Borrelia* spp., but negative for *B. miyamotoi*, were considered positive for *B. burgdorferi* s.l. 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 rection conditions were identical, except annealing which was performed at 55°C, and 40 total reaction cycles were used. The outer reaction conditions for *B. odocoilei* included an initial denaturation at 95°C for 10 min, followed by 40 cycles at 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 *B. 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 sole 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 solitary 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

## Tick collection

A total of 25 *I. scapularis* adults (14 females, 11 males) were collected by flagging low-level vegetation and brush.

## Molecular analysis

The infection prevalence of the four tick-borne zoonotic pathogens is provided as follows: *Borrelia burgdorferi* sensu lato, 17/25 (68%); *Babesia odocoilei*, 10/25 (40%); *Babesia microti*, 2/25 (8%); and *Anaplasma phagocytophilum*, 3/25 (12%).

In the present tick-pathogen study, we had six co-infections and, of these, four were *B. burgdorferi* sensu lato—*B. odocoilei* co-infections. One was a *B. burgdorferi* s.l.—*B. microti* co-infection, and one was a *B. burgdorferi* s.l.—*A. phagocytophilum* co-infection.

Clearly, 25 ticks were adequate to detect the 4 pathogens, and determined their prevalence. Statistical analysis was not applicable.

With the presence of 4 different tick-borne zoonotic pathogens, we reveal why patients do not get better with standard Lyme disease treatments.

In this hyperendemic area, people have a higher risk of becoming infected with tick-borne zoonotic pathogens when bitten by an *I. scapularis* tick. Whenever transovarial transmission occurs in forest-dwelling areas, there can certainly

be an increase in the infection prevalence of *B. odocoilei*.

## Discussion

The origin of piroplasmids and ticks date back to ~300 Ma. Since then, *B. odocoilei* has honed its genome to adapt to certain *Ixodes* spp. (i.e., *I. scapularis*), and current weather conditions in a temperate zone. In order to get a better understanding of the pathophysiology of *B. odocoilei*, we reviewed the scientific literature of veterinary *Babesia* and *Plasmodium falciparum* malaria. Once human babesiosis caused by *B. odocoilei* establishes in the body, and becomes chronic, it can become a life-threating infection.

### Alternative modes of transmission of *Babesia odocoilei*

*Babesia odocoilei* has various means of transmission, via natural and man-made portals. Within *I. scapularis* ticks, *B. odocoilei* is stored in the salivary glands just posterior to the base of the hypostome. When the tick bites, *B. odocoilei* is promptly transferred to the host. *Babesia odocoilei* may also be transmitted by blood transfusion [32], and by organ transplantation [33]. Also, it may be transmitted by maternal-fetal transmission [34-37].

### *Babesia odocoilei* employs highly specific survival strategies

*Ixodes scapularis* females store *B. odocoilei* kinetes in their salivary glands. When a *B. odocoilei*-infected *I. scapularis* bites its host, kinetes are transmitted very quickly. Transmission is similar to the virus of Powassan Disease Virus, which, likewise, is stored in the tick salivary glands. This transmission can occur in 15 minutes. On the other hand, the midgut can hold an amalgam of pathogens (e.g., *B. burgdorferi* s.l.) and, upon a tick bite, crosses the epithelium (midgut outer tissue). These pathogens slowly migrates to the salivary glands and, subsequently, are expelled via the hypostome. The journey of *B. burgdorferi* s.l. is longer, and slower that *B. odocoilei*. If *B. odocoilei* is commingling with certain pathogens, such as Powassan virus, *B. odocoilei* may be transmitted promptly. When the *I. scapularis* tick begins to feed, the kinetes transmute into infected sporozoites. These sporozoites convert quickly to infected trophozoites. Gradually, they advance to infective merozoites, and they change the pathophysiology of the arterial system of the host. At the same time, fibrinogen converts to fibrin, and adheres to the walls of the blood vessels (endothelium). This process, called cytoadherence, permits fibrin to adhere to the endothelium [38]. Synonymously, fibrin combines with uninfected red blood cells (uRBCs) and infected red blood cells (iRBCs). All together (fibrin, uRBCs, and iRBCs) propagates fibrin-bonded entanglements in capillaries—this process fulfills sequestration [39].

### Fibrin-bonded entanglements block capillaries

In time, capillaries and venules become partially or completely occluded. Such blockage depletes the body of oxygen and nutrients. A sequestering *Babesia* sp., such as *B. odocoilei*, propagate fibrin-bonded entanglements, especially in the brain, which has the smallest capillaries. Tiny capillaries in the brain, intestines, and lungs exacerbate cytoadherence and sequestration. Remarkably, cytoadherence and sequestration are highly effective modes of evading the spleen and the circulating immune system and, thus, produce chronic infection. Deep-seated fibrin-bonded entanglements are the key obstructive mechanisms, and are the persistence factor in sequestering *Babesia* spp. Both Lyme disease and human babesiosis can be persistent in the human body.

### Transovarial transmission in *Ixodes scapularis*

*Babesia odocoilei* belongs to the *Babesia* sensu stricto lineage, which is characteristic of *Babesia*

spp. that facilitates transovarial transmission (gravid female to eggs to larvae) [39,40]. Kinetes pass through these developmental life stages without a *B. odocoilei*-infected host. During each molt, *B. odocoilei* then undergo transstadial passage (larva to nymph &/or nymph to adult) [40,41]. In the case of *B. odocoilei*-infected *I. scapularis*, larvae, nymphs and gravid females do not need an infected host to acquire infection because they are already infected. This babesial piroplasmid passes easily to the next generation. Infective gravid females can perpetuate *B. odocoilei* for several generations.

Coincidently, scientists collected a *B. odocoilei*-infected *I. scapularis* from a North American porcupine, *Erethizon dorsatum*, in the same geographic location as the current established population [20].

## Songbirds involved in an established population

Neotropical songbirds play an integral role in the wide dispersal of songbird-transported ticks [9,15-21]. At the collection site, ground-foraging songbirds play key roles in the enzootic cycle of tick-borne zoonotic pathogens (Figure 1). Scientists have detected *B. burgdorferi* s.l., *B. odocoilei* and *A. phagocytophilum* in brachial blood of passerines during the nesting period [42]. When fully engorged larvae and nymphs drop from their hosts, they must go through a molt of 5 to 8 wk before they are ready to take the next blood meal from avian or mammalian hosts, including humans. Thus, when people become infected, the enzootic cycle becomes an epizootic cycle. *Ixodes scapularis* juveniles and females can transmit tick-borne zoonotic pathogens, such as co-infections of *B. burgdorferi* s.l. [1] and *B. odocoilei* [2,3]. During bimodal spring and fall migration, tick-infected songbirds move across borders. During fall migration, neotropical songbirds can transport juvenile *I. scapularis* as far south as the Caribbeans, Central America, and the northern part of South America.

When an *I. scapularis* larva or nymph, which has parasitized passerines, becomes positive for *B. odocoilei*, we suggest that these juveniles acquired the infection from the blood of the bird. However, these juvenile *I. scapularis* can also become infected with *B. odocoilei* via transovarial transmission. Since the present collection site in Lanark County is a May-June nesting area, this woodland habitat is part of the enzootic transmission cycle of *B. burgdorferi* s.l., *B. odocoilei*, and *A. phagocytophilum*.

## Co-infections and polymicrobial treatment

Co-infections and polymicrobial treatments are complex. First, ticks need to be tested for tick-borne zoonotic pathogens. If a person has a co-infection of *B. burgdorferi* s.l. and *B.*

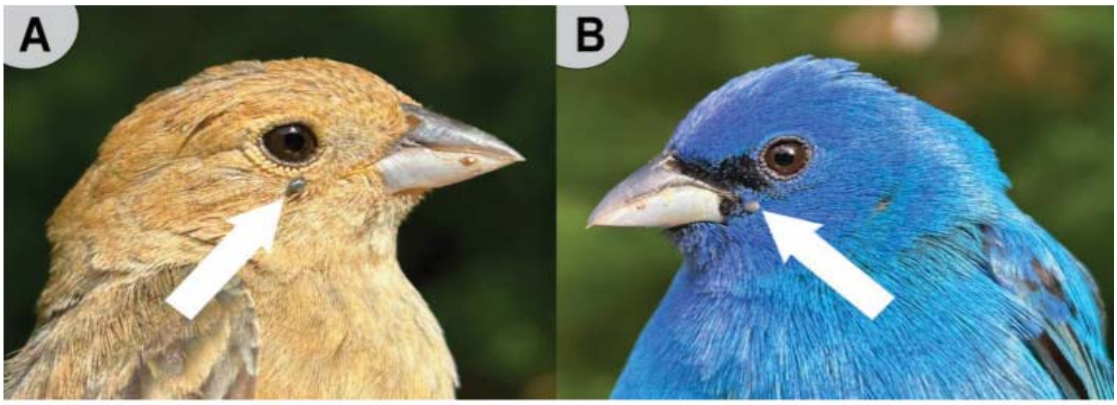


**Figure 1** A). Indigo Bunting, female parasitized by an *Ixodes scapularis* nymph. B). Indigo Bunting, male parasitized by *Ixodes scapularis* nymphs. Photo credits: Nancy Furber.

*odocoilei*, the later will block the recovery of a Lyme disease patient doing solo Lyme disease treatment [43]. *Borrelia burgdorferi* s.l. is a spirochetal bacterium, whereas *B. odocoilei* is a red blood cell piroplasmid. In the present study, we encountered 6 co-infections and, of these, four were *B. burgdorferi* s.l. and *B. odocoilei* co-infections. These two tick-borne zoonotic pathogens require much different treatment than a solo regimen.

In the pioneer days of Lyme disease, public health officials decided that they would label hard-to-treat Lyme disease cases as Post-Treatment Lyme Disease Syndrome (PTLDS). In the present study, we provide a bona fide explanation of why PTLDS does not exist. Recognizing co-infections and polymicrobial infection epitomizes the essential need for quick action, and thorough testing for tick-borne zoonotic pathogens.

## *Babesia odocoilei*-infected *Ixodes scapularis* females induce public health risk

*Ixodes scapularis* can remain infective *B. odocoilei* for generations without feeding on infected hosts. In counterpoint, both *B. microti* (a non-sequestering *Babesia* sp.) and *B. burgdorferi* s.l. are not transmitted transovarially. Therefore, uninfected *I. scapularis* larvae must feed on an infected host to become infected nymphs.

*Babesia odocoilei*-infected *I. scapularis* females can greatly amplify *B. odocoilei* within a humid, arboreal habitat. When a gravid *I. scapularis* female lays a mass of 1,000 eggs on the forest floor, a new generation of *B. odocoilei*-infected ticks may be borne. Since these larvae can start the transmission of *B. odocoilei* one step sooner via transovarial transmission, the prevalence increased greatly. As a result, *B. odocoilei*-infective *I. scapularis* amplifies a severe epidemiological health risk, especially for people who visit forest-dwelling habitats during temperate months. Because of their minute size (0.75 mm), larvae are extremely difficult to detect [21]. In other words, *I. scapularis* larvae hatched from a mass of eggs laid by a *B. odocoilei*-infected female, will create a danger zone, especially for picnickers or children

**Table 2:** Symptoms associated with human babesiosis caused by *Babesia odocoilei.*

| | | |
|---|---|---|
| **Early-onset of symptoms: may occur in the first 6 months** | | |
| unremitting fatigue | sweats (especially at night) | cognitive impairment |
| sluggishness in head | ischemic (slow blood circulation) | lack of reading comprehension |
| amplified thirst | sleep disturbance/insomnia | clumsiness/poor balance |
| pronounced inflammation | anxiety, tearfulness | headaches/head pressure |
| numbness in fingers/face | constipation, lethargic bowels | anhedonia (inability to feel joy) |
| increased blood pressure | dysphoric (intense uneasiness) | urinary hesitancy |
| difficult remembering | unsteady gait/lack of balance | air hunger, shortness of breath |
| cognitive impairment | hampered reading retention | chills, heat and cold intolerance |
| fluctuation of emotions | sore eyes/unexplained pain | disorientation/delirium |
| nausea/abdominal pain | periods of being in a daze | liver ache (especially at night) |
| muscle ache/joint pain | pathogen-induced depression | irritability/ aggression/rage |
| panic attack/feel scared | weird/wild dreams | loss of interest in hobbies |
| **Late-onset of symptoms: typically occur after 6 months** | | |
| muscle weakness | poor stamina | major depression |
| dizziness/blurred vision | chronic encephalitis | coma/ stroke/seizures |
| dysautonomia, nervousness | memory loss, dementia | white matter hyperintensities |
| peripheral neuropathy | severe hemolysis | motion sickness/difficulty walking |
| intolerance to physical activity | suicidal/homicidal ideation | dyslexia (trouble reading/writing) |
| intolerance of mental exertion | nightmares, hallucinations | restless legs/muscle spasm/shakes |

resting on the ground. Without tick repellant, people frequenting a shady, wooded area in temperate months (above zero ˚C, and no snow cover) encounter a heighten health hazard *via* a tiny tick bite.

## Symptoms of human babesiosis caused by *Babesia odocoilei*

Based on morbidity, we categorize the symptoms of human babesiosis caused by *B. odocoilei* into two sequential categories, namely early-onset and late-onset (Table 2).

As cytoadherence and sequestration develop, human babesiosis patients have severe exertional intolerance, chronic encephalopathy and, in some cases, have fatal outcomes (Daniel Cameron, MD). Since *B. odocoilei* is a sequestering *Babesia* sp., fibrin-bonded entanglements will occlude capillaries and post-capillary venules by forming self-contained, self-perpetuating colonies. Notably, this disease is recalcitrant to treat [2,3]. On the contrary, *B. microti* is a non-sequestering *Babesia* sp., and is normally less difficult to treat. Some patients are diagnosed with dementia until they are properly re-assessed with human babesiosis caused by *B. odocoilei* [2,3].

## Clinicians have no explanation

Alarmingly, when a clinician is not familiar with human babesiosis caused by *B. odocoilei*, they label the patient with a medley of different diseases, such as chronic fatigue syndrome, psychotic depression, POTS, mast-cell activation syndrome, fibromyalgia, multiple sclerosis, Alzheimer's disease, dementia, unexplained autoimmune disease issues, psychiatric illnesses, schizophrenia, Rasmussen's syndrome, and more. Anyone with a known or suspected tick bite often assume that the pathogen is Lyme disease. Unfortunately, this default treatment only works part of the time with *I. scapularis* tick bites. Scientists have found that *I. scapularis* is just as apt to be infected with *B. odocoilei* as *B. burgdorferi* s.l. [9].

Sequestering *Babesia* spp. are notorious for clogging capillaries, and gradually decelerating the function of mitochondria—the body's energy factories. Because of the ongoing presence of *B. odocoilei* toxins, production of ATP is greatly hindered. Physical and mental activity quickly exhaust the available ATP. During rest and sleep, humans rejuvenate somewhat with their ATP, but after waking, activity promptly utilizes it. This is the pattern of fatigue in patients with human babesiosis caused by *B. odocoilei* [2,3,9,44].

Similar to Lyme disease, piroplasmosis becomes chronic when it is fully established throughout the body [45]. The arbitrary time for pronounced symptoms is considered to be 6 months. Based on extensive medical experience, a prolonged antibabesial therapy is required for a cure. Clinicians typically use an antibiotic (i.e., doxycycline) to treat tick bites. Unfortunately, this default treatment only works haphazardly. For multiple reasons, a single-dose doxycycline does not work [46]. Not only does *B. burgdorferi* s.l. persist in deep-seated niches, it resides in many body tissues. *Borrelia burgdorferi* s.l. has at least 4 diverse forms, plus a biofilm. *Borrelia burgdorferi* s.l. sequesters in scar tissue, cartilage, brain, eye, and neuronal and glial cells [47-49]. On the other hand, sequestering *Babesia* spp. (i.e., *Babesia canis*, *B. odocoilei*) elude single-dose doxycycline. A co-infection, which has *B. odocoilei*, does not respond to doxycycline. In one particular tick study, four different pathogens were detected in a single *I. scapularis* adult [50]. Tick-borne polymicrobial infections are common in patients, but infrequently reported [51]. When patients have polymicrobial infections, clinicians must use a multiplex antimicrobial regimen. Unquestionably, *B. odocoilei* stalls Lyme disease recovery.

At autopsy, researchers recently detected *B. odocoilei* in the brain of a 2-yr-old boy residing

in Ontario, Canada and, tragically, he had a fatal outcome [52].

Medically, *B. odocoilei* is a parasite that doxycycline does not treat. Symptoms of human babesiosis caused by *B. odocoilei* can be wide-ranging (Table 2), and difficult for healthcare practitioners to recognize. Testing with a reputable health laboratory is fundamental. A missed or delayed diagnosis can be costly and life-destroying [53].

## Conclusion

*Babesia odocoilei* is one tick-borne zoonotic pathogen that can be passed from one generation to the next. Transmission is done by storing *B. odocoilei* in the ovaries of an *I. scapularis* female and, subsequently, in rudimentary ovaries of future developmental life stages. When a person is bitten by an *I. scapularis* tick, they should have the tick tested for tick-borne zoonotic pathogens, especially *B. burgdorferi* s.l. and *B. odocoilei*. *Ixodes scapularis* has a phenomenal ability to multiply *B. odocoilei* infectivity, especially in established populations.

We show that there is no such medical condition as PTLDS. A tick bite with tick-borne zoonotic pathogens can result in debilitating and fatal outcomes. Whenever someone is having an organ transplantation, patients should have testing for tick-borne pathogens. Likewise, when a donor gives blood, and the recipient receives a blood transfusion, both recipient and donor bloods need to be tested for tick-borne pathogens. If a pregnant person is bitten by a tick during pregnancy, the mother needs to have the cord blood and the placenta tested for tick-borne zoonotic pathogens.

Clinicians must have a polymicrobial regimen to treat polymicrobial infections in people. Patients with human babesiosis caused by *B. odocoilei* typically exhibit the biomarkers of dementia, and this zoonosis becomes an energy-draining and brain-altering disease. This flagship study re-affirms that anyone can acquire *Borrelia burgdorferi* sensu lato, *Babesia odocoilei*, *Anaplasma phagocytophilum*, and *Babesia microti* from *Ixodes scapularis*. Early treatment is vital for full recovery. Moreover, *B. burgdorferi* and *B. odocoilei* are often co-infections, and provides further evidence that Lyme disease can be persistent.

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### Ethical consideration

Ethical approval is not required to flag for ticks.

### Authors' contribution

Conceptualization and design: JDS and CMS. Collection and methodology: JDS. Formal analysis: JDS and CMS. Drafting of manuscript: JDS and CMS.

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

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

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