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Molecular Detection of *Anaplasma phagocytophilum*, *Babesia odocoilei*, and *Borrelia burgdorferi* Sensu Lato in *Ixodes scapularis* Ticks Collected in Veterinary Clinics in Southern Wellington County, Ontario, Canada

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Abstract

Tick-borne zoonotic diseases are sinister afflictions to mankind world-wide. A total of 96 adults of the blacklegged tick, *Ixodes scapularis*, were collected in southern Wellington County. Using molecular analysis, three pathogens were detected, namely *Borrelia burgdorferi* sensu lato (s.l.), 24/96 (25%), *Babesia odocoilei*, 15/96 (16%), and *Anaplasma phagocytophilum,* 1/96 (1%). A single co-infection consisting of *B. burgdorferi* s.l. and *A. phagocytophilum* was also detected. We report the first tick-host-pathogen study in southern Wellington County. Overall, 16 established populations were discovered. If clinicians only test and treat patients for the Lyme disease bacterium, they miss 40% of the tick-borne zoonotic infections.

Keywords

Blacklegged Tick, *Ixodes scapularis*, Established Population, Tick-Borne Zoonotic Diseases, *Borrelia burgdorferi* Sensu Lato, *Babesia odocoilei, Anaplasma phagocytophilum,* Pathogen, Veterinary Clinics

1. Introduction

Tick-borne zoonotic pathogens represent an increasing public health risk world-wide. In North America, an established population of blacklegged ticks, *Ixodes scapularis* (Acari: Ixodidae), is based on at least 6 ticks of a developmental life

stage or at least two of the three host-seeking life stages in a single collection period [1]. *Ixodes scapularis* is known to harbor at least seven pathogens, namely *Anaplasma phagocytophilum* [2], *Babesia microti* [3], *Babesia odocoilei* [4] [5], *Borrelia burgdorferi* sensu lato (s.l.) complex [6], *Borrelia miyamotoi* [7], *Ehrlichia muris eauclairensis* [8], and the virus of Powassan Virus Disease [9]. These microorganisms are all pathogenic to humans. Of these 7 pathogens, the most recently discovered pathogen is *B. odocoilei* [4] [5]. *Ixodes scapularis* larvae, nymphs and females are ectoparasite during the tick bite, but after the blood meal, *B. odocoilei* is an endoparasite of red blood cells within the arterial system.

Babesia odocoilei (Apicomplexa: Piroplasmidae: Babesiidae) is an intracellular, red blood cell parasite. This piroplasmid is a sequestering *Babesia* sp., that is pathogenic to humans [4] [5]. This single-celled microbe is found across North America [10], the United Kingdom [11], and the European Union [12]. *Babesia odocoilei* is a virulent cousin of *Plasmodium falciparum*, the causative microorganism of malaria. This sequestering *Babesia* sp. is unique because it enables transovarial transmission (gravid female to eggs to larvae) [13] [14]. In Southern Ontario, *B. odocoilei* has been detected in unfed larvae of *I. scapularis* in the Rouge Valley, Toronto and at the Toronto Zoo [15]. These unfed larvae were infected with *B. odocoilei*.

Co-infections and polymicrobial infections are common in patients but infrequently reported [16]. Notably, four different pathogens have been detected in a single *I. scapularis* adult collected in Wisconsin [17]. In eastern and central North America, *I. scapularis* is the primary tick vector of *B. odocoilei* [18]. In California, *B. odocoilei* has been detected in the western blacklegged tick, *Ixodes pacificus*, in California [19]. Likewise, in B.C., *B. odocoilei* has been found in *I. pacificus* and *I. scapularis* [20].

Natural reservoir hosts of *B. odocoilei* are cervids (*i.e.*, white-tailed deer, *Odocoileus virginianus*) [21]-[23]. Bovids (*i.e.*, desert bighorn sheep, *Ovis canadensis nelsoni*) are also reservoirs [24] [25].

Babesia odocoilei has been detected in avian-transported larval and nymphal *I. scapularis*. Songbirds (Order: Passeriformes; Suborder: Passeri) play a key role in the wide dispersal of songbird-transported ticks especially during bidirectional migrations [26]-[34]. Additionally, *B. odocoilei* has been detected in the brachial blood of songbirds during the summer nesting period [33]. When juvenile *I. scapularis* molt to the next stage, transstadial passage (larva to nymph &/or nymph to adult) of *B. odocoilel* occurs [14]. In one cross-Canada study, scientists found that the natural ratio of *B. odocoilei* to *B. microti* in *I. scapularis* adults was 60:1 [10].

The present study set out 1) to determine the endemicity of *I. scapularis* in southern Wellington County, and 2) to find the prevalence of *Borrelia burgdorferi* s.l. *Babesia odocoilei*, *Babesia microti*, *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, and *Bartonella* spp., in this area.

2. Materials and Methods

2.1. Tick Collection

Veterinarians and veterinary technicians collected ticks during the fall stage of the

bimodal, questing activity period of *I. scapularis* adults. Tick-collecting kits were hand-delivered to veterinary clinics in southern Wellington County in late September 2025. These kits were collected in early December 2025. The collection period for this study was approximately 2.5 months. Each kit had a crate of 6 micro tubes containing 95% ethyl alcohol. Each micro tube had a label to record the collection data (host, geographic location, date collected). An Olympus SZX16 stereoscopic microscope was used to identify the ticks. Using taxonomic keys, tick nomenclature and tick identification were determined and confirmed [35]-[37]. Of note, the spirochetal bacterium, *B. burgdorferi* s.l. was previously detected in Centre Wellington [37].

2.2. Molecular Analysis

All DNA extractions and PCRs were completed by Geneticks Inc. (Uxbridge, ON). Adult ticks were bisected longitudinally and homogenized by bead beating 400 µl of DNA/RNA shield (Zymo Research, Irvine, CA) with a mix of 2.3 mm and 0.1 mm Zirconia/Silica beads (BioSpec Products, Bartlesville, OK). Samples were subjected to two subsequent runs of 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**. All samples were tested for the presence of *Borrelia burgdorferi* s.l. complex., *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 (PCR Biosystems, London, UK). Subsequently, 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 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, Mississauga, ON). To interpret qPCR results, the following algorithm was used: samples that tested positive for both *Borrelia* spp., and *B. miyamotoi* were considered positive for *B. miyamotoi*. 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.

Quality control measures were implemented at the diagnostic laboratory. Both engineering and processing controls were employed to identify and prevent aerosol contamination, and assure assay quality. Ticks were tested in batches of 15 - 20 samples at a time, with workspaces and instruments decontaminated with 0.5% sodium hypochlorite solution between each batch. Physically separated Biosafety cabinets (Class 2A) were used for DNA extraction, PCR master mix formulation, and sample loading. For each batch, no template control (NTC) reactions using Buffer TE pH 8.0 instead of template DNA were included for each PCR assay em-

ployed. A DNA extraction and amplification control were included for each sample targeting the "Folmer region" of the CO1 gene (https://pubmed.ncbi.nlm.nih.gov/7881515/). Any samples that failed to amplify the Folmer region, or any batches where the NTC was positive, were considered non-viable, and discarded or repeated. For any qPCR samples where the Cq value was above 35, results were verified using an nPCR assay targeting the *Borrelia* 23s intergenic spacer unit (https://pmc.ncbi.nlm.nih.gov/articles/PMC4001108/). All nPCR assays were confirmed using a proprietary multiplex qPCR developed by ThermoFisher (Mississauga, ON) for use at Geneticks Inc.

Table 1. Primers and Probes used to detect pathogens in *Ixodes scapularis* ticks.

Genus/Species	Gene	PCR Type	Primer Name	Sequence (5'-3')	Amplicon Size	Reference
Borrelia spp.	23s IGS	qPCR	Bb23Sf	cgagctcttaaaagggcgatttagt	75	[38]
			Bb23Sr	gcttcagcctggccataaatag		
			Bb23SProbe	FAM-apatgtggtagacccgaagccgagtgc-ECLIPSE		
Borrelia miyamotoi	flaB	qPCR	flaBf	agcacaagcttcatggacattga	102	[39]
			flaBr	gagctgcttgagcaccttctc		
			flabProbe	FAM-tgtggtgtcaaatcaggatgaagca-ECLIPSE		
Anaplasma phagocytophilum	msp2	Nested PCR	AnaP44OutL1-F	GTAGAAGAAACCGCCCTAAT	850	[40]
			AnaP44OutL1-R	TCTATGTTGGTTTGGATTACAG		
			MSP3F	CCAGCGTTTAGCAAGATAAGAG	334	[41]
			MSP3R	GCCCAGTAACAACATCATAAGC		
Babesia microti	18s rRNA	Nested PCR	Babs1	CTTAGTATAAGCTTTTATACAGC	238	[42]
			Bab4	ATAGGTCAGAAACTTGAATGATACA		
			Bab2	GTTATAGTTTATTTGATGTTC	155	
			Bab3	AAGCCATGCGATTCGCTAAT		
Babesia odocoilei	18s rRNA	Nested PCR	Bab306R_RCF	TTTCTGCGTCACCGTATT	331	[43]
			BabGenInR2	ACGACGGTATCTGATCGTCT	311	[40]
			odo563	CCGTATTTTGACTTTTGTCGACTGT		
			BabGenInR1	TCTGATCGTCTTCGATCCCC		
Bartonella spp.	RibC	Nested PCR	RibC-1F	CGGATATCGGTTGTGTTGAA	309	[44]
			RibC-1R	CATCAATRTGACCAGAAACCA		
			RibC-2F	GCATCAATTGCGTGTTCA	185	
			RibC-2R	CCCATTTCATCACCCAAT		

Note: Reference numbers for **Table 1** are as follows: *Borrelia* spp. [38], *Borrelia miyamotoi* [39], *Anaplasma phagocytophilum* [40] [41], *Babesia microti* [42], *Babesia odocoilei* [43] [40]; *Bartonella* spp. [44].

3. Result

3.1. Tick Collection

In all, 96 *Ixodes scapularis* ticks were collected at 19 veterinary clinics in southern Wellington County during the fall questing period (26 September to 5 December

2025). For the purpose of this study, Centre Wellington was combined with the townships in southern Wellinton County. In total, 96 *I. scapularis* ticks were collected from 75 hosts (domestic dogs, *Canis lupus familiaris*, 55; domestic cats, *Felis catus*, 13; humans, *Homo sapiens*, 4; and horses, *Equus caballus*, 3).

One *I. scapularis* female had a co-infection of *B. burgdorferi* s.l. and *A. phagocytophilum*.

Relapsing fever (*Borrelia hermsii*) was not detected; it is normally found in far-western North America.

3.2. Molecular Analysis

The infection prevalences of the four tick-borne zoonotic pathogens was *Borrelia burgdorferi* s.l., 24/96 (25%), *B. odocoilei*, 15/96 (16%) and *A. phagocytophilum1*/96 (1%). *Babesia microti*, *Borrelia miyamotoi*, *Bartonella* spp. were not detected.

Overall, we found 16 areas in southern Wellington County with the number of established populations of *I. scapularis* as follows: Erin, 7; Guelph/Eramosa, 2; Guelph, 1; Puslinch, 1; and Centre Wellington, 5.

4. Discussion

The epicentre of an established population may be less than a hectare, but white-tailed deer and songbirds can play a pivotal role in wide dispersal of *B. odocoilei*-infected *I. scapularis* ticks. In this study, both domestic dogs and domestic cats played a focal role; they had outside activity. The Ontario Provincial Police state that the number of deer strikes in the study area increased from the previous year. Since deer are reservoirs of *B. odocoilei*, there is most likely an increase in the prevalence of *B. odocoilei* In *I. scapularis*. Patients are becoming increasingly dissatisfied that clinicians are side-stepping the diagnosis of tick-borne zoonotic diseases. To better understand the pathophysiology of *B. odocoilei*, we gleaned information from the scientific literature on veterinary *Babesia* and *Plasmodium falciparum* malaria.

4.1. Development of Fibrin-Bonded Entanglements

When *I. scapularis* larvae, nymphs, and females parasitize a person, kinetes quickly convert to sporozoites, and then change to trophozoites, and onward to infective merozoites. During this process, fibrinogen converts to fibrin in the blood stream, and adheres to the endothelium cells. This attachment process is called cytoadherence [45]. In synonymy, fibrin binds with uninfected red blood cells (uRBCs) and infected red blood cells (iRBCs). All together (fibrin, iRBCs. uRBCs), these fibrin-bonded entanglements set up in the capillaries and post-capillary venules, and begin the implementation of sequestration [46]. With these preliminary steps, pathogenesis is underway. As a result, patients have reduced circulation, and encounter unrelenting fatigue.

Once a capillary is clogged, the fibrin-bonded entanglement becomes a self-

perpetuating, and self-protective housing. In time, multiple occlusion containments throughout the body are able to shut down the body systems.

Sequestering *Babesia* spp. are noted for clogging capillaries, and slowing the function of mitochondria—the body's energy factories. Because of the perpetual presence of *B. odocoilei* toxins, production of ATP is dramatically reduced. Mental and physical activities greatly exhaust the availability of ATP. During sleep and rest, patients rejuvenate somewhat with ATP, but after awakening, activity promptly utilizes ATP. Thus, ongoing fatigue is a common pattern in patients with human babesiosis caused by *B. odocoilei* [4] [5] [10] [20] [47].

4.2. Persistence and Chronicity of the Lyme Disease Bacterium

Both persistence and chronicity of *B. burgdorferi* s.l. and *B. odocoilei* have been confirmed in the human body. In particular, *B. burgdorferi* s.l. has diverse forms, and hides in different deep-seated tissues (*i.e.*, scar tissue, bone, eye, brain, neuronal and glial cells) [48] [49]. Left untreated or undertreated, this bacterium becomes chronic [48] [49]. Many studies and animal models show that persistence is the direct cause of *B. burgdorferi* s.l. [49]. Some pathologists and clinicians consider persistence and chronicity are one-in-the-same. We believe that persistence leads to chronicity. The authors currently have a list of 362 peer-reviewed scientific articles on the persistence and chronicity of *B. burgdorferi* s.l. These citations confirm persistence and chronicity of Lyme disease caused by *B. burgdorferi* s.l. Similarly, the authors have documented persistence and chronicity of human babesiosis caused by *B. odocoilei* in 15 citations. Both *B. burgdorferi* s.l. and *B. odocoilei* are pleomorphic and have diverse forms.

4.3. Comprehensive Testing and Treatment

Accurate testing and treatment is of utmost importance. Based on the present study, 40% of the *I. scapularis* infections would be missed if clinicians only tested and treated the Lyme disease bacterium. In the case of *B. odocoilei*, delayed testing and treatment could result in a lifetime of suffering—an education and career loss—an agonizing future.

4.4. Zoonoses in Established Populations of *Ixodes scapularis*

People from urban areas (e.g., Kingston, Toronto, Montreal), where *I. scapularis* are endemic, take their tick-infested dogs to rural areas, such as southern Wellington County. In the new area, fully engorged *I. scapularis* drop to the ground, and molt to the next life stage. They then parasitize other hosts, including people. In the fall, residents may return to the same area to enjoy parkland and fall colours, and acquire the next developmental life stage of the tick. When they bring their tick-infested dog on their return visit to the same area, the likelihood of forming an established population increases.

Epidemiologically, in Huronia, scientists found that 71% of the *I. scapularis* adults, which were parasitized by domestic dogs and feral cats, were infected with

B. odocoilei [30]; none was infected with *B. burgdorferi s.*l. Researchers of the present study, found that 16% of the fall-collected *I. scapularis* were infected with *B. odocoilei*. Symptoms are listed as early-onset and late-onset stages in Table 2.

4.5. Symptoms of Human Babesiosis Caused by *Babesia odocoilei*

Table 2. Symptoms related to human babesiosis caused by *Babesia odocoilei*.

Early onset of symptoms that commonly occur in the first 6 months		
cognitive decline	unrelenting fatigue/low stamina	poor balance/clumsiness
being in daze	lack of reading comprehension	ischemic (slow blood circulation)
extra thirst	sleep disturbance/insomnia	mood changes, ambivalence
anxiety, fearfulness	profound inflammation	head pressure/headaches
urinary hesitation	constipation, lethargic bowels	numbness in fingers/face
difficult remembering	unsteady gait, lack of balance	anhedonia (inability to feel joy)
cognitive impairment	air hunger/shortness of breath	hampered reading retention
panic attack/feel scared	sore eyes/unexplained pain	liver ache (especially at night)
fluctuation of emotions	disorientation/delirium	headaches/head pressure
joint pain/ muscle ache	pathogen-induced depression	irritability/aggression/rage
weird/wild dreams	sluggishness in head	loss of interest in hobbies
nausea/abdominal pain	hyperacoustic (sensitive to noise)	chills/heat & cold intolerance
Late-onset of symptoms that typically occurs after 6 months		
muscle weakness	ticked off, disgusted	dyslexia (trouble reading & writing)
chronic encephalitis	dizziness/blurred vision	vasculopathy in blood vessels
dementia/memory loss	nervousness/dystonia	white matter hyperintensities
severe hemolysis	peripheral neuropathy	difficult walking/motion sickness
coma/seizures/stroke	intolerance to physical activity	suicidal/homicidal ideation
hallucination/nightmare	intolerance of mental exertion	restless legs/muscle spasms/shakes

Note: Clinicians are often labelling human babesiosis caused by *B. odocoilei* with an assortment of different diseases, such as ME/chronic fatigue syndrome, Alzheimer's disease, fibromyalgia, multiple sclerosis, POTS, dementia, neuropsychiatric disease, psychotic depression, and more.

4.6. Chronicity of Human Babesiosis Caused by *Babesia odocoilei*

Some healthcare providers consider persistence and chronicity one-in-the-same. Because of the adaptability and persistence of *B. odocoilei*, chronicity is very prevalent. Babesiosis sneaks in slowly as the parasitemia level increases, and this piroplasmid becomes established in capillaries as fibrin-bonded entanglements infecting more and more red blood cells. In the early stage, when the parasitemia level of *B. odocoilei* is building, it can cause considerable fatigue, muscle ache, body pain, and disorientation. In the later stage, dementia, cognitive impairment, major depression, and difficulty walking can occur. As a persistent infection, this newly-discovered, babesial zoonosis becomes long-lasting and deep-rooted in the

human arterial system. In time, this intracellular parasite instigates a lingering, life-long, and incurable disease.

4.7. Ticks Are Nature's Unsanitary Syringes

Babesia odocoilei is stored in the salivary glands of the *I. scapularis* tick. At the initial stage of the tick bite, kinetes leave the salivary glands, and surges forward into the hypostome, and directly into the blood stream of the host. In *I. scapularis* females, *B. odocoilei* is stored in both the salivary glands and the ovaries. A fully engorged gravid female can transmit *B. odocoilei* to humans and then deposit a mass of infected eggs on the forest floor. One month after egg laying starts, these eggs can become *B. odocoilei*- infected larvae that promptly start host-seeking activities. This area becomes a danger zone because this leaflitter habitat is covered with a thousand *B. odocoilei*-infected larvae. Any child that crawls or lays on the ground in one of these endemic areas is sure to contract human babesiosis caused by *B. odocoilei* [4] [5]. Whenever a fully engorged, gravid *I. scapularis* female is infected with *B. odocoilei*, this babesial infection will be maintained to the next generation. As long as a fully engorged, gravid female is infected with *B. odocoilei*, a new generation of *I. scapularis* infected with *B. odocoilei* will be maintained and, therefore, *B. odocoilei* can be propagated, ad infinitum. This area where eggs were laid presents a genuine health risk in the woods.

4.8. Transovarial Transmission in Toronto

Although we did not find *B. odocoilei*-infected *I. scapularis* larvae in southern Wellington County, as our study focused on adult *I. scapularis*. Milne *et al.* [15] collected unfed *I. scapularis* larvae in Toronto, and these larvae were infected with *B. odocoilei*. Transovarial transmission (gravid female to eggs to larvae) is ongoing in Toronto. Flagging around oak trees in early August is a potential way to collect them.

If *I. scapularis* females are infected with *B. odocoilei*, they typically pass infective kinetes to the eggs and, subsequently, to hatching larvae, and onward to suitable hosts (e.g., songbirds, humans). The larvae do not have to bite an infected host to become infected. Transovarial transmission is a unique way to transmit *B. odocoilei* for many generations.

Because larvae are very tiny (0.75 mm), they are hard to see. Children laying on the ground in wooded areas are prime targets for *B. odocoilei*-infected *I. scapularis* larvae. This scenario can generate a public health crisis.

4.9. Migratory Songbirds Disperse Ticks

Neotropical passerines play a pivotal role in the widespread dispersal of *I. scapularis* larvae and nymphs [50] [51]. These long-distance migrants transport ticks as far south as the northern part of South America [50] [51]. Not only are passerines heavily involved in the wide dispersal of songbird-transported tick, these ground-foraging passerines are implicated in the enzootic cycle of at least 7 different path-

ogens. When people are bitten, they are involved with an epizootic cycle. Of note, agrologists recently discovered *I. scapularis* parasitizing avian and mammalian hosts in B.C. [52] [53]. During northward spring migration, scientists have collected juvenile *I. scapularis* on ground-frequenting songbirds, nation-wide, as far north, and as far west as northwestern Alberta [26] [27] [50] [51].

Notably, scientists detected *B. burgdorferi* s.l., *B. odocoilei*, and *A. phagocytophilum* in brachial blood of songbirds during the nesting period [33]. Juvenile *I. scapularis* have the potential to contract tick-borne zoonotic pathogens from songbirds. In fact, researchers have found that the American robin, *Turdus migratorius*, is a reservoir-competent bird species that transmits the Lyme disease bacterium to juvenile *I. scapularis* [54] [55].

4.10. Treatment Obstacles

Penetration of capillaries that are occluded is the major obstacle in the treatment of human babesiosis caused by *Babesia odocoilei* [4] [5]. Alarmingly, they can form self-contained, self-perpetuating hideaways that can live on indefinitely. Fibrinolytics (e.g. nattokinase, serrapeptase, lumbrokinase) loosen fibrin from the endothelium, and iRBCs, and uRBCs. Fibrinolytics allow antibabesials to act more effectively. Because human babesiosis caused by *B. odocoilei* is persistent, this sequestering *Babesia* sp. is very recalcitrant to treat. Early testing and prompt treatment are paramount.

5. Conclusion

We discovered 16 established populations of *I. scapularis* ticks in southern Wellington County. Collectively, these established populations harbour *B. burgdorferi* s.l., 24/96 (25%); *B. odocoilei*, 15/96 (16%); and *A. phagocytophilum*, 1/96 (1%). These microorganisms are all pathogenic to humans. Clinicians must be schooled in these pathogens, and ticks removed from patients must be tested for these three tick-borne zoonotic diseases. Whenever patients have been visiting a wooded area during temperate months (above 0˚C, no snow cover), they must realize that they face questing ticks—an environmental hazard. Human babesiosis caused by *B. odocoilei* is pathogenic to humans. Based on this flagship study, clinicians who only test for the Lyme disease bacterium, would miss 40% of the tick-borne zoonotic pathogens. Clinicians who diagnose and treat tick-borne zoonotic diseases must have a full understanding of the pathology of this apicomplexan parasite and, subsequently, realize that these microorganisms can cause an energy-draining, insidious zoonoses.

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

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Conflicts of Interest

The authors have no conflicts to declare.

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