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Human Babesiosis Caused by *Babesia odocoilei*: Hiding Behind a Mask

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Abstract

Background: Human babesiosis is a tick-transmitted, zoonotic disease caused by a single-celled, intraerythrocytic parasite of the genus *Babesia*. In North America, *Babesia odocoilei* is the predominant *Babesia* species, and has considerable medical and economic dysfunction. Sequestering *Babesia* spp. form occlusions and blockage in capillaries, and are recalcitrant to treat.

Methods: This international human babesiosis study encompassed 113 participants from Canada and the USA. A professional phlebotomist drew blood from each participant. The human study protocol was approved by WCG IRB, an international ethical review board. Nucleic acid detection was used to carry out *Babesia* identification.

Results: Since participants had been taking antibabesials before blood draw, nucleic acid detection of *B. odocoilei* was thwarted. Most participants had an array of clinical symptoms associated with human babesiosis. On the consent form, the most common symptoms were unrelenting fatigue, muscle or joint ache, sleep disturbance, brain fog, and slow/impaired cognition.

Conclusion: *Babesia odocoilei*-infected *I. scapularis* are playing havoc with patients' lives. The 18S rRNA gene is not stable when the patient has been on babesial treatment prior to blood draw. Nucleic acid detection results are unreliable when patients have previously taken antibabesials.

Keywords: Human babesiosis; Intraerythrocytic parasite; Molecular identification; *Babesia odocoilei*; Zoonosis; Fibrin-bonding entanglements; Fibrolytics; Antibabesials; International study

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Abbreviation

WCG IRB: Western Copernicus Group Institutional Review Board; CITI: Collaborative Institutional Training Initiative; FDA: Food and Drug Administration; ATP: Adenosine triphosphate; RBCs: Red blood cells; iRBCs: infected red blood cells; uRBCs: uninfected red blood cells; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic Acid; bp: base pair; FISH: Fluorescence *in situ* hybridization; PCR: Polymerase chain reaction; REB: Research Ethics Board

Introduction

Human babesiosis (human piroplasmosis), a tick-transmitted zoonosis, is caused by an intraerythrocytic parasite of the genus *Babesia* (Apicomplexa: Piroplasmida: Babesiidae). These single-celled parasites inflict a major impact on certain vertebrates, especially humans. The common mode of transmission is a tick bite [1]; however, transferal can occur by blood transfusion [2], organ transplantation [3], and maternal-fetal transmission [4]. The blacklegged tick, *Ixodes scapularis* (Acari: Ixodidae), is the primary vector of *Babesia odocoilei* in North America. When a *B. odocoilei*-infected *I. scapularis* bites, and takes a blood meal, it transmits sporozoites into the blood stream. *Babesia* sporozoites attack red blood cells, and promptly change to infective trophozoites (aka piriforms). Phylogenetically, *B. odocoilei* is a cousin of *Plasmodium falciparum*, the causal organism of malaria. It is estimated that there are at least 111 valid *Babesia* spp. around the world [5]. Clinical manifestations of human babesiosis lie on the continuum from mild to severe, and patients can die [6].

Globally, *Babesia* species that are known to be pathogenic to humans include *B. crassa*-like [7], *B. divergens* [8], *Babesia divergens*-like MO-1 [9], *B. duncani* [10], *B. microti* [11], *B. motasi* [12], *B. odocoilei* [13], *Babesia* spp. XXB/HangZhou [14], *Babesia* sp. TW1 [15], *Babesia* spp. CA1, CA3,

CA4 [16], and *B. venatorum* [17]. *Babesia* spp. divide into 10 basic clades based on phylogenetic lineage [18,19].

Babesia odocoilei has been described in several U.S. states and Canadian provinces, including California [20], Indiana [21-23], Iowa [24], Maine [22,23], Massachusetts [21-23], Michigan [25], Minnesota [24], Montana [24], New Hampshire [26], New York [26,27], Ohio [24], Oklahoma [19,28], Pennsylvania [26,29,30], Texas [26,31,32], Virginia [33], and Washington [34], and Wisconsin [22,23]. In Canada, researchers have reported *B. odocoilei* in Saskatchewan [35], Ontario [36-40], Quebec [36,37,39,40], Nova Scotia [39], and British Columbia [41].

Songbirds (Order: Passeriformes) play a crucial role in the wide dispersal of juvenile *I. scapularis* that are infected with tick-borne zoonotic pathogens [36-39,42-48]. People can contract human babesiosis wherever *B. odocoilei*-infected *I. scapularis* are dispersed. Notably, acarologists, ornithologists, and molecular biologists recently detected *B. odocoilei* in the blood of passerines during the nesting season [40]. These songbirds were all parasitized by juvenile *I. scapularis* that were infected with *B. odocoilei* [36-39,42-48]. Migratory songbirds transport *B. odocoilei*-infected *I. scapularis* larvae and nymphs across the Canada-U.S. border. Not only do songbirds have cross-border movement during spring and fall migration [36-39], white-tailed deer transport *B. odocoilei* across the border, either on foot, or by swimming. Travelers also cross the Canada-U.S. border with companion animals infested with *I. scapularis* ticks that may be infected with *B. odocoilei*. These ixodid ectoparasites carry and transmit at least 6 tick-borne zoonotic pathogens that inflicted untold suffering, and can be fatal [6,9].

The primary reservoir of *B. odocoilei* are cervids (i.e., white-tailed deer, *Odocoileus virginianus*; wapiti, *Cervus elaphus canadensis*), and perpetuate *B. odocoilei* in the environment [28,31,33]. They are also hosts of all 3 motile, development life stages of *I. scapularis*, especially adults. *Babesia odocoilei*-infected *I. scapularis* females exhibit transovarial transmission (female to eggs to larvae) [18,19,49]. This transmission mode occurs within *Babesia* sensu stricto members (Clade X, true *Babesia*) [18,19]. In contrast, *B. microti* belongs to *Babesia* sensu lato (Clade 1), which can only be transmitted by transstadial passage (larva to nymph or nymph to adult). Epidemiologically, when a gravid, *B. odocoilei*-infected female lays her eggs, and hatch, these larvae will be infected.

Certain *Babesia* species, such as *B. odocoilei*, have sequestration. The sequestration process begins when *B. odocoilei* sporozoites enter the blood stream, and circulating fibrinogen (a soluble protein) converts to fibrin. The fibrin combines with iRBCs and uRBCs to form fibrin-bonding entanglements. These entanglements occlude and obstruct capillaries and venules. At the same time, the entanglements adhere to the endothelium forming a blockage. In direct contrast, *B. microti* does not exhibit sequestration, and is relatively easy to treat.

This study aimed to determine whether *B. odocoilei*, found in *I. scapularis* ticks, is also present in humans. Since nucleic acid detection is required to confirm the genus and species of piroplasmids, we employed molecular techniques for species identification.

Materials and Methods

Ethical clearance

Ethical approval for this study, reference #20233072, was attained from WCG IRB Canada; approval was acquired before the study commenced. The review by WCG IRB Canada has been conducted in accordance with Division 5 regulations of Health Canada, the Tri-Council Policy Statement, International Conference on Harmonization (ICH) Guidance for Industry - E6 Good Clinical Practice: Consolidated Guidelines, and U.S. CFR Title 21 Parts 50 and 56 and CFR Title 45 Part 46. The present study has followed basic tenets laid out in the Declaration of Helsinki, the Nuremberg Code, the Belmont Report, and the CITI program. WCG IRB, the parent company, is registered with the FDA.

Clinic protocol

Participation in the *Babesia* Study: An open invitation to attend a one-day *Babesia* clinic was extended opportunistically to the public *via* networking. Participants who suspected they might have human babesiosis came to the clinic of their own free will. On arrival at the healthcare center, they were informed about the human *Babesia* study, and asked to read the input and consent form. If they wished to participate, they filled out the form. They posed any questions relating to the study. We informed participants that diagnosis, treatment, and dosage are outside our mandate for this *Babesia* study. Therefore, the efficacy of antibabesials was not expounded upon in this study.

Collecting blood samples: Clinics were held in four locations, namely southwestern Ontario, southwestern Quebec, south-central Quebec; and Pennsylvania. At each location, a professional phlebotomist drew venous blood in a separate room for privacy. Two 4-mL tubes of whole blood EDTA were drawn from each participant. The vacutainer™ (vacuum tube, lavender top) which contains EDTA is a preservative that keeps RBCs separated, and prevents them from clotting. Participants were entered into a two-step, de-identification process (first, by a 3-letter code and, second, by a number code) to secure privacy of information. Blood samples were put directly into a cooler. When each blood sample was taken, participants had no further responsibilities to the study.

A courier transported samples to the laboratory in a special temperature-controlled cooling box that maintains the temperature between 2°C and 8°C.

Laboratory protocol

***Babesia* detection:** For biosafety measures, conventional personal protective equipment was used in the laboratory. DNA was extracted from human blood using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol for blood. DNA extracts were stored at −20°C until the PCR was performed. The BJ1 (5′-GTC-TTG-TAA-TTG-GAA-TGA-TGG-3′) and BN2 (5′-TAG-TTT-ATG-GTT-AGG-ACT-ACG-3′) primers were used to amplify the 18S rRNA gene of *Babesia* using PCR conditions previously described [13]. Protocol modifications included a greater volume of erythrocyte DNA (~5 ng/μL). In order to intensify the reaction mixture, 5 μL of DNA was used instead of 2.5 μL, whereas 2.5 μL of H_2O was applied instead of 5 μL of water. In total, not more than 25 μL was utilized for the PCR reaction mixture. The resulting amplicons were visualized on 1.8% agarose gel containing GelStar nucleic acid stain (Lonza, Rockland, ME, USA), and the *Babesia* amplicons that were 420 bp to 490 bp in length.

Results

Adhering to the institutional review board's legal-regulator, organizational, and disciplinary professional requirement, we assembled key statistical information. The consent forms of 113

participants were assessed for human babesiosis symptoms. The symptom response, by count, is listed in descending order as follows: Fatigue (106), muscle or joint aches (102), sleep disturbance (97), brain fog (91), slow/impaired cognition (84), anxiety or depression (75), intestinal problems (73), night sweats (65), headaches (pressure) (64), numbness in fingers (48), chills (46), and intensified thirst (44). Most participants had these symptoms that are indicative of human babesiosis caused by *B. odocoilei*.

Participants displayed a wide array of clinical symptoms (13,34). Most participants had *B. odocoilei* symptomology, whereas a few had none. Common symptoms on the consent form included perpetual fatigue, sweats (especially at night), sleep disturbance, numbness in fingers (at night), muscle or joint aches, intensified thirst, constipation, intestinal problems, environmental sensitivities, cold intolerance, headache (pressure), anger, anxiety/depression, brain fog, slow/impaired cognition. *Babesia odocoilei* causes reduced cognition, and produces cerebral pathophysiology.

From the consent form, 25 (76%) of 33 participants, who had FISH (tests for genus *Babesia*) conducted by commercial laboratories, were positive for *Babesia*. These results indicate that these participants were infected with a *Babesia* species.

On the consent form, 26 (87%) of 30 participants who had done an immunoassay serology test previously, were positive for *B. duncani*. These facts indicate that the participants previously had evidence of positivity for *B. duncani*. However, *B. duncani* had never been detected in *I. scapularis* in the study area. These serology results from commercial laboratories indicate cross-reactions. These findings indicate cross-reactivity between *B. duncani* and *B. odocoilei*.

Although the 18S rRNA gene resembled *Babesia* in 5 participants, it lacked concordance genetically with the type strain of *B. odocoilei*. When double-checked, the samples that were previously considered positive for *Babesia* were re-run through BioEdit software. We found the homologies were closer to strange piroplasmids. Because participants had been taking antibabesials before blood draw, standard nucleic acid detection methodology to test *Babesia* species was stymied.

Participants listed a wide selection of herbals and anti-*Babesia* drugs they used. Many antiparasitic regimens provided a temporary relief for participants, but none of the treatments listed provided a cure. In some cases, participants had added a fibrinolytic enzyme, by capsule, to help disentangle and loosen fibrin-bonding entanglements from capillaries and venules, but we could not assess the effect.

One participant who had definitive proof of *B. odocoilei* in a previous study [13], was false negative in the current study due to having antibabesials prior to blood draw.

Discussion

We describe a sequestering *Babesia* sp. in this international human babesiosis study. Celebrities, healthcare professionals, and the public are contracting this tick-borne zoonosis, and are not able to function at school or work. Oftentimes, some patients are labelled with other conditions and maladies. The present study shows that human babesiosis caused by certain *Babesia* species is widespread. In order to find a well - rounded solution, researchers, clinicians and patients must deal with zoonosis in a concerted, forthrigt manner. In order to get a clear understanding of human babesiosis caused by *B. odocoilei*, we gleaned authoritative, background knowledge on sequestering *Babesia* species from the veterinary literature.

Low parasitemia levels

In the present study, low-level parasitemia of *Babesia* in human blood in the present study could have resulted from several factors. First, participants had such a low parasitemia level that PCR did not detect this cagey, babesial piroplasmid. Second, participants had been taking antibabesials (pharmaceutical drugs and herbals), and had suppressed and distorted the level of *B. odocoilei* infection. Third, fibrin-bonding entanglements that are composed of fibrin, iRBCs, and uRBCs were densely impacted in capillaries and venules, and *Babesia* was not accessible in venous blood. *Babesia*-altering chemicals, such as antibabesials masked the actual *Babesia* infection. Even though participants tested negative in the present study, they may, in fact, have been false negatives. Self-perpetuating entanglements hold iRBCs in microvasculature of sequestering *Babesia* species. In contrast blood from songbirds tested positive for *B. odocoilei* using nucleic acid detection [48]. As well, *I. scapularis* do not take antibabesials, and are consistently positive for *B. odocoilei via* nucleic acid detection [36-39,46-48].

Supportive *Babesia* serology and FISH testing

In this study, serology and FISH tests provided reassuring evidence of *Babesia*. Overall, 26 (87%) of 30 patients who had a serology test, were positive for *B. duncani*. And yet, *B. duncani* has never been found in *I. scapularis* ticks collected from avian and mammalian hosts in the study area [30,35-40,42-48]. In addition, 25 (76 %) of 33 participants were positive for *Babesia* by FISH testing. After six year of testing hundreds of *I. scapularis* ticks collected in the sampling area, none tested positive for *B. duncani*. The overwhelming majority of *I. scapularis* ticks were positive for *B. odocoilei*. Since some participants were positive using FISH and/or serology tests, we extrapolate that they were infected with a *Babesia* sp.

Fibrin-bonded entanglements occlude capillaries and venules

When *B. odocoilei*-infected *I. scapularis* take a blood meal from its host, infective sporozoites enter the RBCs where they develop into trophozoites [18]. During the infective process, fibrin strands bind iRBCs and uRBCs to each other, and to the endothelium (cytoadherence) [50,51]. As the infective *Babesia* propagate, fibrin-bonding entanglements obstruct capillaries and venules [53,54]. Sequestering *Babesia* spp. can complete their life cycle within these entanglements and, thus, remain isolated from the spleen and the circulating immune system [51-54]. On the contrary, occlusions obstruct capillaries and venules throughout the body, especially the brain, which has the smallest capillaries.

Sequestering *Babesia* spp. (i.e., *B. canis*, *B. odocoilei*) are capable of cytoadherence [53], and these pathophysiological mechanisms are highly effective means of evading the spleen and the circulating immune system [54]. Sequestering *Babesia* spp. can complete their life cycle within the fibrin-bonding entanglements [50-53]. On the contrary non-sequestering spp. (i.e., *B. microti*) circulate freely within the body, and are typically trapped by the spleen (an immunological barrier), and attacked by macrophages in the circulating immune system. A babesial vaccine is not feasible in sequestring *Babesia* because it would not penetrate self-perpetuating, fibrin-bonded entanglements. Moreover, vaccines suppress the immune system [55].

Since fibrin-bonding entanglements sequester in smaller capillaries (i.e., brain, intestines, lungs), this apicomplexan merozoites promote cognitive impairments and cerebral pathophysiology. At the same time these occlusions obstruct capillaries and venules throughout the host's body inflicting sequestration and, thus, depriving tissues of oxygen and nutrients. Mental and physical activities quickly deplete the available ATP (molecule that provides energy) [56,57].

In the present study, the majority of participants who had previous serological antibody testing were positive for *B. duncani*. Biographically, *B. duncani* has never been detected in hundreds of *I. scapularis* ticks collected in the study area that have been tested during the past six years [30,39,42-48]. *Babesia odocoilei* is prevalent in many arboreal parts of temperate Canada and the USA, especially where cervids are indigenous, and birds drop *B. odocoilei*-infected *I. scapularis* larvae and nymphs. In one Canadian study, the prevalence of *B. odocoilei*-infected *I. scapularis* adults was 20% [37]. In another prevalence study *I. scapularis* adults collected from dogs and cats in the Huronia area, had a prevalence of 71% for *B. odocoilei* [38]. In addition, the ratio of *B. odocoilei* to *B. microti* was 41 to 1 [39]. As well, *B. microti* was not found in Ontario or Quebec [36,37,39]. The vast majority were positive for *B. odocoilei* not *B. duncani* or *B. microti*. In Pennsylvania, the ratio of *B. odocoilei* to *B. microti* was 23 to 1 [30]. Unquestionably, *B. odocoilei* is the predominant *Babesia* sp. Unlike *B. microti*, which is a non-sequestering *Babesia* species, *B. odocoilei* instigates sequestration in capillaries and venules and, therefore, is one of the most difficult *Babesia* spp. to treat successfully. Based on contemporary medical information and pharmaceutical drugs that are currently available for clinical use, early treatment is obligatory to mitigate the parasitemia level, and speed up a cure. Based on empirical clinical experience, *B. odocoilei* requires prolonged antibabesial therapy [13].

Fibrinolytic enzymes and antibabesial prior to blood draw

Some participants used fibrinolytics (i.e., lumbrokinase, or nattokinase, or serrapeptase) plus antibabesials prior to blood draw to disentangle and release fibrin-bonding entanglements. Although fibrinolytics and antibabesials were not mentioned on the consent form, participants had employed them. *Babesia odocoilei* produces a nuance of pathophysiological changes in the body when self-perpetuating entanglements lodge in capillaries and venules. In fact, researchers found that the number of merozoites in fibrin-bonding entanglements in capillaries was much greater than in circulating venous blood up to 25× more [53]. Circulating fibrinogen convert to fibrin, and forms fibrin-bonding entanglements that cause sequestration in mammals, such *B. canis* in dogs [53].

Determining the causal organism

After tick removal, it is essential to have ticks identified. If the tick is either western blacklegged tick, *Ixodes pacificus* or *I. scapularis*, it is vital to have the tick tested for tick-borne zoonotic pathogens. Depending on the pathogens detected, the most efficacious antimicrobials can be administered. Congruent with other studies *B. odocoilei* is the most common *Babesia* species [13,36-39,47,48]. Of note, 3% of the nymphal and adult *I. pacificus* tested in California were infected with *B. odocoilei* [20].

When antibabesials are taken before blood draw, the structure of *Babesia* is altered in the venous blood (which goes to the laboratory). Genetically, the 18S rRNA gene is not stable when the patient has been on babesial treatment. Untreated erythrocytes that thrive and propagate in the self-sustaining entanglements continue to cycle unaltered. Patients continue to experience symptoms of human babesiosis, and gradually become dysfunctional.

Symptoms associated with human babesiosis caused by sequestering *Babesia*

In advanced cases of chronic human babesiosis, patients can have any combination of the following symptoms, including long-standing fatigue, exertional intolerance, poor balance, shaky limbs, cognitive dysfunction, dysphoric (anguish), unending inflammation, muscle stiffness, unsteady walking, clumsiness, slow urination, dizziness, profound/wild dreams, nightmares, absent-mindedness, pathogen-induced depression, suicidal ideation, angry/harmful outbursts, progressive dementia, white matter hyperintensities ischemia, severe encephalitis, stroke and coma. In some cases, patients become bedridden and disabled. Some human babesiosis patients succumb to death [6,9,58-61]. Because *B. odocoilei* sequesters in capillaries and venules, this zoonosis is recalcitrant, especially in treatment-resistant babesiosis [13,51]. Due to sequestration, human babesiosis becomes a persistent and chronic infection. Patients get labelled with conditions and maladies including fibromyalgia, ADHD, chronic fatigue syndrome, oxidative stress, psychosomatic depression, "long-COVID," bipolar disorder, dementia, white matter hyper-intensities, Alzheimer's disease, autism, multiple sclerosis, myalgic encephalomyelitis, irritable bowel syndrome, autoimmune disorder, personality disorders and mast-cell activation syndrome.

Single-cell parasite treatment strategy

For human babesiosis treatment, participants used a wide-ranging spectrum of antibabesial medications (i.e., artesunate, ivermectin, primaquine, tafenoquine). Some added a fibrinolytic enzyme to help release fibrin-bonding entanglements from capillaries and venules. Fibrinolytics soften and release fibrin attached to self-perpetuating entanglements from the endothelium. The combination of antibabesials and fibrinolytics helped greatly in treating patients with a sequestering *Babesia* sp. Some used pulse-therapy (i.e., 3 d on, 3 d off) to give the body a pause to clear toxins and detritus. The jury is out on how long it takes to completely eradicate sequestering *Babesia* spp. from the human body.

Increased public health risk due to transovarial transmission in *Ixodes scapularis*

Babesia odocoilei-infected *I. scapularis* females exhibit transovarial transmission (female to eggs to larvae) [18,19,49]. When *B. odocoilei*-infected *I. scapularis* females lay eggs, they hatch to infective larvae in late July and, subsequent, after a blood meal, molt to infective nymphs, and then after another blood meal, molt to infective adults. *Babesia odocoilei* infected *I. scapularis* ticks can remain infective for generations without feeding on infected hosts. These lifelong carriers enhance an increase risk to the public. In contrast, *B. microti* only employs transstadial passage, and larvae and nymphs must feed on infected hosts to become infected.

The fact that *I. scapularis* larvae can transmit *B. odocoilei* intensifies an epidemiological problem for the human population. As people expand into woodland habitats and recreational areas (i.e., parks, and campgrounds) exposure to *B. odocoilei*-infected *I. scapularis* escalates. A gravid *I. scapularis* female can lay 1000 infective eggs on the forest floor, and intensify a much greater health risk to the public. Because of the larva's minute size (0.75 mm) finding them is extremely difficult. As well, infective larvae extend the questing season, especially in established populations with deer. Based on transovarial transmission of *B. odocoilei*, people are just as apt to contract human babesiosis as Lyme disease. For tick prevention, outdoor adventurers should

eschew I. scapularis habitats whenever the temperature is above zero, and there is no snow cover. Outdoors people must use a tick repellant, and do a full-body tick check after outings. Putting dry clothes in the dryer on the "High" setting for 4 min is a prudent strategy to kill ticks.

Based on animal models, *B. burgdorferi* transmission can occur in <16 h [62]. *Borrelia burgdorferi* is stored in the midgut, and later transmitted to the salivary glands, and then to the hypostome [63]. In contrast, *I. scapularis* ticks, which store the virus of Powassan Virus Disease in the salivary glands, can transmit this infective virus to the host within 15 min [64,65]. Likewise, *B. odocoilei* ookinetes are stored in the salivary glands adjacent to the entrance of the hypostome and, at tick attachment, can promptly transmit infective sporozoites. The salivary glands surrounding the salivary ducts empty directly into the hypostome salivarium and, therefore, this feature makes rapid passage of infective sporozoites into the blood stream [66].

Sequestering *Babesia* species are recalcitrant to treat. In fact, *B. odocoilei*, an apicomplexan hemoparasite has evolved and outfoxed modern medicine for 15 to 20 million years [67].

Conclusion

Based on deductive reasoning, we conclude that many participants were infected with *B. odocoilei*. We know *I. scapularis* is the primary vector of *B. odocoilei* in the study area. In addition, *B. odocoilei* is the predominant *Babesia* sp. in an ever-increasing, continent-wide zoonosis. White-tailed deer are reservoirs of *B. odocoilei*, and are a source of infection throughout the investigated area and beyond. Songbirds widely disperse *B. odocoilei*-infected *I. scapularis* throughout northeastern North America. Passerine birds carry *B. odocoilei* in their blood, and they do not consume antibabesials. Nucleic acid detection works well in testing songbird blood. From FISH testing, 25 participants were positive for *Babesia*, which indicates that they were infected with a *Babesia* sp. Similarly, from serology testing, 26 participants were positive for *B. duncani*; however, *B. duncani* has never been detected in *I. scapularis* ticks within the survey region. Cross-reactivity between *B. duncani* and *B. odocoilei* is clearly evident. *Babesia odocoilei* sequesters in self-perpetuating, fibrin-bonding entanglements in capillaries and venules. When venous blood is drawn by venipuncture, damaged 18S rRNA is not recognized by nucleic acid detection. When antibabesials are taken before blood draw, DNA of the 18S rRNA gene gets altered and degraded. DNA degradation was apparent. Instituted by logical reasoning, we conclude that numerous participants were infected with human babesiosis caused by *B. odocoilei*.

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