

## ENVIRONMENTAL INVESTIGATION FOLLOWING THE FIRST HUMAN CASE OF BABESIOSIS IN TENNESSEE

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**ABSTRACT:** Babesiosis is an emerging tick-borne zoonotic disease in the United States caused by *Babesia* parasites. In 2009, the first case of babesiosis was documented in Tennessee. Environmental investigation at the reported site of tick exposure included collection of ticks and specimens from eastern cottontail rabbits (*Sylvilagus floridanus*) and white-tailed deer (*Odocoileus virginianus*) that were tested for piroplasmids by molecular and serologic methods. One hundred and sixty-six *Ixodes scapularis* ticks and biological samples from 8 rabbits and 5 deer were collected. *Ixodes scapularis* were PCR positive for *Babesia odocoilei* (n = 7, 4%) and *Theileria cervi* (n = 24, 14%). Deer were seropositive for *B. odocoilei* and PCR positive for *T. cervi*. Rabbits were seropositive for *B. odocoilei* and *Babesia* sp. MO1, and 1 rabbit was PCR positive for *Babesia* sp. MO1. In summary, zoonotic *Babesia* sp. MO1 infection in rabbits is reported here for the first time in Tennessee as well as infection of deer and *I. scapularis* ticks with 2 other piroplasmids of veterinary importance.

*Babesia* spp. are apicomplexan piroplasmids that are of medical and veterinary importance worldwide and can cause severe disease in humans, domestic animals, and wildlife (Homer et al., 2000; Kjemtrup et al., 2000). Transmission of *Babesia* spp. is through the bite of an infected ixodid tick (Oliveira and Kreier, 1979; Herwaldt et al., 1995; Florin-Christensen and Schnittger, 2009). In the United States, *Babesia microti* is the most common human-infecting species, and it is maintained in nature by rodents (primarily white-footed mice, *Peromyscus leucopus*) and black-legged tick, *Ixodes scapularis* (Oliveira and Kreier, 1979). In addition to tick transmission, babesiosis can be acquired through blood transfusions, and babesiosis is the most commonly reported transfusion-associated tick-borne infection in the United States (Chamberland et al., 1998). The majority of transfusion-associated babesiosis cases have been caused by *B. microti*, but a second species, *Babesia duncani*, has also been transmitted via blood transfusions (Herwaldt et al., 1997, 2002; Gorenflot et al., 1998). Between 1997 and 2007, there were 9 deaths due to transfusion transmitted babesiosis reported to the U.S. Food and Drug Administration (Gubernot et al., 2009).

Babesiosis is considered an emerging disease in the United States, and the incidence and geographic range of infections have been increasing since the first human case was documented in Massachusetts in 1969 (Fitzpatrick et al., 1968). Human babesiosis in the United States is primarily caused by *B. microti*. The majority of these cases occur in the northeastern United States, though cases have also been reported from the northern Midwest (Herwaldt et al., 1995; Gorenflot et al., 1998). *Babesia microti* is thought to be less virulent than *Babesia divergens*, the primary agent of babesiosis in Europe, and often causes infection that is asymptomatic or associated with mild nonspecific symptoms (Hatcher et al., 2001). In endemic areas, splenectomized individuals are at the highest risk for severe infection,

though elderly and immunocompromised persons are also at a higher risk for severe disease (Gorenflot et al., 1998).

*Babesia* spp. can cause substantial morbidity and mortality in domestic animal and wildlife hosts globally. As an example, *Babesia odocoilei* has been found in a variety of mammals, including white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), farmed reindeer/caribou (*Rangifer tarandus*), bighorn sheep (*Ovis canadensis*), and mule deer (*Odocoileus hemionus*) (Waldrup et al., 1989; Goff et al., 1993; Thomford et al., 1996; Holman et al., 2003). Though the pathogenicity of *B. odocoilei* is thought to be variable, it can result in hemolytic anemia, anorexia, and death in free-ranging animals. It is not known to cause illness in humans (Perry et al., 1985).

In this report, we describe the environmental investigations to incriminate possible vectors and reservoir hosts of *Babesia* following the first documented case of babesiosis in Tennessee (E. M. Mosites et al., unpubl. data). The case was a 43-year-old immunosuppressed patient who lived in central Tennessee and had a history of idiopathic thrombocytopenic purpura with therapeutic splenectomy. *Babesia* parasites were noted on blood smear, and the patient was PCR positive for a *Babesia*. The patient resided on 2–3 acres in a rural Tennessee town and worked in a metropolitan area. He had not traveled outside of Tennessee in several years (neither nationally nor internationally). Plausible exposures reported prior to illness onset included contact with animals, ticks, and tick habitat while hunting white-tailed deer at 1 location, a 150-acre farm in a nearby county. No tick bites were recalled, although the patient had regular contact with ticks on harvested deer, and ticks were found on him during hunting season.

### MATERIALS AND METHODS

#### Wildlife and tick collection

The field investigation focused on a single rural location in Tennessee where the patient could have had tick exposure and included the collection of wildlife and tick specimens. Symptom onset for the patient was in December 2008, and so sampling began in November 2009 to collect tick species active during that time of year. Potential animal reservoir species for *Babesia* spp. that were collected did not include mice since *B. microti* was excluded as the potential agent of infection for the Tennessee case. Blood was collected from the body cavity of 5 hunter-killed white-tailed deer in EDTA tubes (Becton Dickinson Vacutainer, Franklin Lakes, New Jersey) in November and December 2009. Blood and spleen specimens were collected from 8 eastern cottontail rabbits in February 2010. All

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TABLE I. Indirect immunofluorescent-antibody (IFA) end titers for samples collected from white-tailed deer in December 2009 and eastern cottontail rabbits in February 2010.

	Date of specimen collection	<i>Babesia</i> / <i>Theileria</i> detected by PCR	IFA titer to <i>B. microti</i>	IFA titer to <i>B. odocoilei</i>	IFA titer to MO-1
White-tailed deer					
D1	Dec. 2009	—	—	1:64	—
D2	Nov. 2009	<i>Theileria</i> sp.	—	—	—
D3	Nov. 2009	—	—	1:128	—
D4	Nov. 2009	<i>Theileria</i> sp.	—	1:128	—
D5	Nov. 2009	<i>Theileria</i> sp.	—	1:64	—
Eastern cottontail rabbits					
R1	Feb. 2010	—	—	—	—
R3	Feb. 2010	<i>Babesia</i> sp. MO1	—	1:512	1:32,768
R4	Feb. 2010	—	—	—	—
R5	Feb. 2010	—	—	1:256	—
R6	Feb. 2010	—	—	1:128	—
R7	Feb. 2010	—	—	—	1:256
R8	Feb. 2010	—	—	1:256	1:32,768

samples were transported on ice to the Tennessee Department of Health Vector-Borne Diseases Laboratory. Adult ticks were collected in November 2009 from hunter-killed deer. Ticks were also collected in November 2009 using flannel drags. We removed ticks from beagle hunting dogs in February 2010. All ticks were identified to species, sex, and life stage based on morphological criteria (Keirans and Litwak, 1989).

#### Wildlife and tick specimen preparation and analysis

Whole blood samples were centrifuged at 2,991 *g* for 5 min to separate and remove the serum. DNA was extracted from deer blood cell DNA and rabbit spleens according to the manufacturer's instructions using the Maxwell 16 Tissue DNA Purification Kit (Promega, Madison, Wisconsin). Ticks were individually homogenized with metal beads and resuspended in 225  $\mu$ l PBS. DNA was extracted from 200  $\mu$ l of the homogenate using a 5 PRIME Manual Ready PCR DNA Column Kit (5 PRIME, Gaithersburg, Maryland) according to the manufacturer's instructions. All serum, blood, spleen, and DNA samples were stored in a  $-80$  C freezer.

Extracted tick DNA was screened for *Babesia* species using a conventional polymerase chain reaction (PCR) with primers PIRO-A and PIRO-B to amplify approximately 400 base pair (bp) region of the 18S rRNA gene (Armstrong et al., 1998). *Theileria* spp. were also amplified by these primers. DNA isolated from eastern cottontail rabbit and white-tailed deer blood samples were screened for *Babesia* using a conventional PCR with primers CRYPTO-F and CRYPTO-R that amplify a wide range of apicomplexans (Herwaldt et al., 2004). PCR products were detected by electrophoresis in a 2% agarose gel containing ethidium bromide. DNA from a white-tailed deer dually positive for *Theileria* sp. and *B. odocoilei* was used as a positive control, and dH<sub>2</sub>O was used as a negative control for all PCR reactions. For species identification, amplicons from positive samples were sequenced with a 3130x1 genetic sequencer (Applied Biosystems, Foster City, California) and BigDye Terminator (Applied Biosystems).

White-tailed deer and eastern cottontail rabbit serum samples were tested for antibodies reactive to *B. microti*, *B. odocoilei*, and *Babesia* sp. MO1 antigens in serial dilutions by IFA. Slides were made in accordance with the method of Chisholm et al. (1978). The *B. microti* strain was prepared in vivo in hamsters (Chisholm et al., 1978), and the *Babesia* sp. MO1 (Nantucket strain) and *B. odocoilei* strains were cultivated and maintained in vitro following Holman et al (Holman et al., 2005). Fluorescein isothiocyanate (FITC)-labeled rabbit anti-deer globulin (Kirkegaard & Perry Laboratories, West Grove, Pennsylvania) and FITC-labeled goat anti-rabbit globulin (Jackson ImmunoResearch Laboratories, Gaithersburg, Maryland) were used at a concentration of

1:50 as the test conjugates. Samples were considered positive at a titer of 1:64.

## RESULTS

At the farm, 166 black-legged ticks (*Ixodes scapularis*) were collected. Seven (4%) *I. scapularis* ticks demonstrated *B. odocoilei* DNA with 100% match to a strain isolated from farmed reindeer and elk in Wisconsin (GenBank AY 237638.1). Twenty-four (14%) *I. scapularis* ticks were infected with *T. cervi*, matching an isolate from a Wisconsin elk (GenBank AY 735135.1).

Five white-tailed deer blood samples and 8 eastern cottontail rabbit blood and spleen samples were collected. Four (80%) of the white-tailed deer serum samples were reactive to *B. odocoilei* antigen. Among the eastern cottontail rabbits, 2 (25%) were reactive to only *B. odocoilei* antigen, 3 (38%) were reactive to only the *Babesia* sp. MO1 antigen, and 2 (25%) were reactive to both *B. odocoilei* and *Babesia* sp. MO1, but titers to the latter were much higher (Table I).

None of the white-tailed deer were positive for *Babesia* spp. by PCR, but 3 (60%) were positive for *Theileria* sp. A single eastern cottontail rabbit was positive for *Babesia* sp. MO1 (Table I) by PCR.

## DISCUSSION

*Babesia* was identified as the cause of a clinical babesiosis case in Tennessee, the first documented case acquired in Tennessee. The patient had not recently traveled or received blood products. He had been splenectomized and was on immunosuppressive therapy, putting him at higher risk of symptomatic infection. The patient suffered symptomatic illness for 7 mo without diagnosis, demonstrating the need for increased clinical recognition of babesiosis even in states where *Babesia* is not believed to be endemic.

Eastern cottontail rabbits collected from middle Tennessee had evidence of infection with *Babesia* sp. MO1, which is the first report of this zoonotic *Babesia* in Tennessee. *Babesia* sp. MO1 has

caused severe disease in humans from Missouri, Washington, and Kentucky, with the case in the Missouri resident resulting in death (Herwaldt et al., 1996). Two of the Tennessee rabbit serum samples were reactive to both *Babesia* sp. MO1 and *B. odocoilei* with much higher titers to *Babesia* sp. MO1. This reaction to *B. odocoilei* could have been the result of cross-reactivity or, potentially, co-infection. *Babesia* sp. MO1 infections have been reported from eastern cottontail rabbits in Nantucket, Massachusetts, and jackrabbits (*Lepus californicus*) and desert cottontails (*Sylvilagus audubonii*) from Texas (Goethert and Telford, 2003; Holman et al., 2005; Yabsley et al., 2006).

*Babesia odocoilei* was the only *Babesia* detected in *I. scapularis* ticks by PCR and white-tailed deer by serology. *Babesia odocoilei* has been found in a variety of wildlife hosts from various regions of the United States (Waldrup et al., 1989; Goff et al., 1993; Thomford et al., 1993; Holman et al., 2003). Evidence of *B. odocoilei* DNA in *I. scapularis* ticks, together with evidence of exposure to the strain in white-tailed deer and eastern cottontail rabbits in this investigation, established a link between the vector and a potential wildlife reservoir. *Babesia odocoilei* has been associated with disease in some hosts (e.g., reindeer), and so is of veterinary importance.

*Theileria cervi*, another wildlife piroplasm, was found in both the white-tailed deer blood DNA and *I. scapularis* ticks. *Theileria cervi* is an intraerythrocytic protozoan parasite of white-tailed deer that is closely related to *Babesia* and was first identified in North America in 1962 (Kreier et al., 1962; Herwaldt et al., 1995). Human disease from *T. cervi* also has not been identified.

Of interest from a public health and entomological perspective, in past tick species studies in Tennessee, *I. scapularis* were collected in low numbers (Moncayo et al., 2010). The large number of *I. scapularis* found during this current environmental investigation was surprising. *Ixodes scapularis* is the vector for other human pathogens, including *Anaplasma phagocytophilum* and *Borrelia burgdorferi*, the etiologic agents of anaplasmosis and Lyme disease, respectively (Orloski et al., 2000). In endemic areas, co-infection with *Babesia* and other pathogens is important to consider in disease severity, diagnosis, and treatment (Homer et al., 2000).

In summary, we report infection of rabbits with *Babesia* sp. MO1, previously unidentified in Tennessee. It is important that health care providers are aware of babesiosis even in non-endemic states, particularly among patients at greater risk of infection such as those that are immunosuppressed. Clinicians should consider babesiosis in the differential diagnosis of patients with febrile illness and hemolytic anemia, regardless of residence or travel history. Increased attention to emerging vector-borne diseases like babesiosis is important from both a clinical and public health perspective.

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