

# The Emergence of Clinically Relevant Babesiosis in Southwestern Wisconsin

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## ABSTRACT

**Objective:** To determine the frequency and characteristics of babesiosis cases, and to assess the impact of the introduction of a tick-borne infection diagnostic panel on babesiosis diagnosis in the region surrounding La Crosse, Wisconsin, where babesiosis in non-travelers was previously rare.

**Methods:** In the spring of 2013, we conducted a point-in-time survey of *Ixodes scapularis* ticks for the presence of *Babesia microti*. We also conducted a retrospective study of all babesiosis cases diagnosed in our health system between January 1, 2004, and November 1, 2013. Finally, we compared the number of babesiosis cases diagnosed during the study period before and after the June 1, 2012, introduction of a tick-borne infection diagnostic panel in our organization.

**Results:** *Babesia microti* was present in 5% of ticks surveyed in our region. Twenty-two cases of babesiosis were diagnosed in our organization during the study period—19 since 2010. The tick-borne infection diagnostic panel was used widely by clinicians, with an attendant increase in babesiosis diagnoses.

**Conclusion:** Babesiosis should be considered endemic in southwestern Wisconsin, and testing should be considered for patients with compatible clinical and laboratory features.

## INTRODUCTION

Babesiosis is a protozoan tick-borne infectious disease that infects erythrocytes of humans and other mammals.<sup>1,2</sup> The vector for *Babesia microti*, the causative agent of babesiosis, is the *Ixodes scapularis* tick, which is also the vector for *Borrelia burgdorferi*, the causative agent of Lyme disease, and *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis. Babesiosis is usually considered to be a relatively mild infection

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of humans characterized by nonspecific symptoms of fever, myalgia, and headache with accompanying laboratory evidence of hemolytic anemia.<sup>1</sup> However, in patients with immunocompromise, particularly asplenia, and in the very young and in individuals aged 50 years and over, it can be a life-threatening infection.<sup>3,4</sup>

The area around La Crosse, Wisconsin, is known to be highly endemic for Lyme disease.<sup>5</sup> More recently, anaplasmosis has emerged in this region.<sup>6</sup> Historically, most clinical cases of babesiosis in Wisconsin originated in the northwest corner of the state, and clinically recognized cases of babesiosis in the southwest region were very uncommon.<sup>2,7-9</sup> A tick prevalence study from 2006 demonstrated no evidence of *B microti* in 100 ticks collected from this region (Figure

1, Tick Collection Site 1).<sup>10</sup> However, in recent years clinicians in our health system reported cases of severe babesiosis requiring hospitalization in patients who had not left western Wisconsin. Therefore, a polymerase chain reaction (PCR) based assay for the detection of *B microti* was developed and a tick survey performed to evaluate for the theretofore unproven presence of babesiosis in our region. The PCR-based assay was made available to clinicians to facilitate the clinical diagnosis of babesiosis. It also was included in a diagnostic panel developed and made available to clinicians to assist in the diagnosis of tick-borne infections (Lyme disease, anaplasmosis, and babesiosis) in patients who seek care for a systemic febrile illness during the tick season.

The purpose of this study was to test our hypothesis that the number of babesiosis cases was increasing in our service area—especially the number of cases unrelated to travel to areas where *I scapularis* ticks were known to carry *B microti*. We also wanted to determine whether a PCR-based tick-borne infection diagnostic panel introduced in our organization on June 1, 2012, is a sensitive and effective test for identification of *Babesia* infection.

## METHODS

In the spring of 2013, we conducted a point-in-time survey of *I scapularis* ticks for the presence of *B microti*. We also conducted a descriptive retrospective cohort study of patients with a babesiosis diagnosis in our health system from January 1, 2004, through November 1, 2013. We then evaluated the utility of a tick-borne infection diagnostic panel, which was introduced in our organization on June 1, 2012. Protocols were reviewed and approved by the Gundersen Clinic, Ltd Human Subjects Committee/ Institutional Review Board prior to the start of the study.

### Tick Collection and Processing

Adult female *I scapularis* ticks were collected from a site in Trempealeau County, Wisconsin, located directly north of La Crosse, by flagging the underbrush (Figure 1, Tick Collection Site 2). Flagging is a common tick collection technique in which a piece of fabric is attached to a dowel, and the investigator sweeps this “flag” through the underbrush. Ticks collected in this manner were transported to the laboratory in 50-ml centrifuge tubes, then stored and processed as described previously.<sup>6</sup> Extracted DNA was stored at  $-20^{\circ}\text{C}$  until tested.

### Study Population and Case Ascertainment

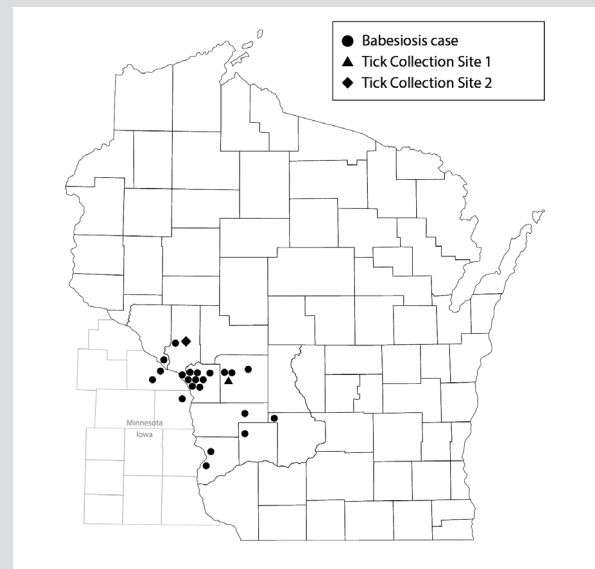
Human cases of babesiosis were identified via multiple methods to ensure complete capture of cases, including searching our medical record database for ICD-9 code 0.88.82 and searching the laboratory database for all blood smears or PCR assays positive for *B microti*. Study inclusion criteria included laboratory confirmation of *Babesia* infection by peripheral smear or buffy coat examination or a PCR assay positive for *B microti* DNA. Patients were excluded from the study if the only laboratory evidence of infection was a positive serologic assay. Medical records were reviewed and pertinent clinical data were recorded. Data collected included demographic data, as well as travel outside the region, known tick bite, and symptoms that might be attributable to a tick-borne infection. Additionally, the results of the tick-borne infection diagnostic panel were tabulated from its implementation on June 1, 2012, through November 1, 2013.

### Patient Samples

From January 1, 2004, until June 1, 2012, a diagnosis of babesiosis was made by identification of parasites on Giemsa-stained blood smears. The laboratory protocol for a complete babesiosis smear included the preparation of 6 thin blood smears that were then examined by 2 separate technicians for at least 300 thin slide fields under  $100\times$  oil immersion. Additionally, a thick blood smear was made by placing 1 to 3 drops of blood onto a slide and spreading manually to an appropriate density, then allowing the slide to air dry overnight prior to staining with Giemsa stain.

Beginning June 1, 2012, a tick-borne infection diagnostic panel was made available in our health system. This panel con-

**Figure 1.** Residence by ZIP Code of 22 Patients Diagnosed With Babesiosis From January 2004 Through November 2013



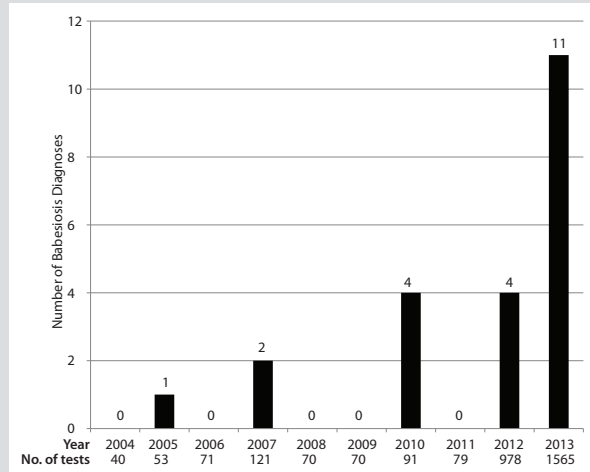
Tick Collection Site 1 represents the location of a study performed in 2006 that did not identify *Babesia microti* in ticks. Site 2 represents the location of a tick prevalence study performed in 2013 that demonstrated 5% prevalence of *B microti*.

sisted of a screening enzyme immunoassay for Lyme disease and a single rapid thin smear analysis for *Anaplasma* and *Babesia* organisms followed by PCR assays for each. More specifically, anaplasmosis and babesiosis diagnostic testing was performed in 2 parts. First blood was collected in an ethylenediaminetetraacetic acid tube, and a smear was created and stained using the SP-1000iHST automatic blood smear. Approximately 300 fields were examined using  $100\times$  oil immersion microscopy for approximately 5 minutes, and the smear was reported as either “no tick-borne organisms seen,” “suspicious for *Anaplasma*,” or “suspicious for *Babesia*.” All samples subsequently underwent *Anaplasma* and *Babesia* PCR testing. The protocol for *B microti* DNA detection is summarized below. The method for *A phagocytophilum* DNA detection by PCR has been described previously.<sup>6</sup> The tick-borne infection diagnostic panel also included the VIDAS IgG and IgM qualitative enzyme-linked fluorescent immunoassay, which was performed according to the laboratory standard operating procedure and interpreted as either negative ( $<0.75$ ), equivocal/indeterminate ( $\geq 0.75 - <1.00$ ), or positive ( $\geq 1.00$ ).

### *B microti* DNA Extraction and Amplification

The DNA was recovered using the commercially available DNA Blood Mini Kit (Qiagen, Valencia, California). Briefly, 1 ml of blood was centrifuged at  $2500 \times g$  for 10 minutes to separate the plasma. A 200- $\mu\text{L}$  volume of red blood cell-enriched blood was then removed and combined with 20  $\mu\text{L}$  of protease and 200  $\mu\text{L}$  of buffer AL. After the suspensions were mixed and incubated

**Figure 2.** Number of Diagnostic Tests for Babesiosis Performed and Number of Cases Diagnosed in Health System in the Region Surrounding La Crosse, Wisconsin by Year



**Table 1.** Results of Diagnostic Tests for *Babesia*, Lyme Disease, and *Anaplasma* in the 22 Patients Diagnosed With Babesiosis in the Region Surrounding La Crosse, Wisconsin<sup>a</sup>

Test Performed	Number of Tests	Tests Positive (%)
Rapid smear for <i>Babesia</i> <sup>b</sup>	15	8 <sup>c</sup> (53)
Complete smear for <i>Babesia</i> <sup>b</sup>	7	7 <sup>c</sup> (100)
PCR assay for <i>Babesia</i>	17	17 (100)
Serology for Lyme disease	20	8 (40)
PCR assay for <i>Anaplasma</i>	19	1 (5)

<sup>a</sup>Use of a tick-borne infection diagnostic panel began in our health system on June 1, 2012.

<sup>b</sup>Performed prior to availability of the tick-borne infection diagnostic panel in our health system.

<sup>c</sup>Includes the number suspicious or positive for *Babesia*.

Abbreviation: PCR, polymerase chain reaction.

at 56°C for 10 minutes, 200 µL of absolute ethanol was added to each sample, and the mixed suspension was transferred into individual columns, washed, and the DNA eluted with 50 µL of buffer AE. The extracted DNA samples were then stored at -20°C until tested.

### Real-time PCR

*B. microti* DNA was detected by real-time PCR using a procedure that targeted a unique sequence with the 18S rRNA gene. Briefly, 5 µL of DNA extracted from patient samples was combined with 20 µL of a master mix that contained 12.5 µL of buffer (10X AmpliTaq Gold buffer, 2.5mM MgCl<sub>2</sub>, deoxynucleoside triphosphates), 4.5 µL of a primer/probe mix comprised of Babf (5'-TCGCGTGCGCTTTATTAGAC-3'), Babr (5'-CCGGCAAAGCCATGCGATT-3'), and Babp-FAM (5'-6-carboxyfluorescein[FAM]-AACCAACCCTTCGGGTAATCGGTG[BHQ1a-FAM-3']), 2.5 µL of exogenous control primer and probe (Life Technologies,

Foster City, California), 0.5 µL of exogenous DNA, and 0.5 µL of AmpliTaq Gold DNA polymerase (1.5 U; Life Technologies). The DNA was then amplified using a real-time thermal cycler (Model 3000P, Stratagene, Cedar Creek, Texas) for 1 cycle at 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 30 seconds, and a final cycle at 25°C for 5 seconds.

### Quality Control

Prior to analyzing the clinical samples, we confirmed the clinical utility of the PCR by amplifying archived DNA extracted from 2 patients with babesiosis diagnoses made by detection of the organisms in blood smears. In addition, we showed that the test could detect as few as 3 DNA copies by resuspending and serially diluting a DNA fragment based on a specific 84-bp sequence within the 18S rRNA gene of *B. microti* isolate Gray (bases 168-185, GenBank accession #AY693840.1) in AE buffer (Qiagen) and testing each dilution. We then used the DNA fragment (50 copies/assay) as a positive control for subsequent runs. In addition, we confirmed that DNA from *Bartonella henselae*, *Plasmodium falciparum*, *A. phagocytophilum*, *B. burgdorferi*, *Borrelia afzelii*, *Borrelia bissetii*, and *Borrelia hermsii* yielded negative findings.

As additional confirmation of our findings, we randomly selected 6 PCR-positive patients and amplified a 425 bp DNA fragment within the *B. microti* 18S rRNA gene as described previously.<sup>1</sup> Briefly, 5 µL of the extracted DNA was combined with a 20.5 µL of master mix that contained 12.5 µL buffer, 1 µL of buffer containing 0.5 µM of primer BJ1 (5'-GTCTTGTAATTGGAATGATGG-3') or BN2 (5'-TAGTTTATGGTTAGGACTACG-3'), 5.5 µL of water, and 0.5 µL AmpliTaq Gold DNA polymerase (1.5 U; Life Technologies). The DNA was amplified under the following conditions: 94°C for 10 minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes, followed by a final extension at 72°C for 5 minutes. We then purified the DNA fragment (QIAquick PCR Purification Kit, Qiagen) and forwarded the products for sequencing (Laragen, Culver City, California). Without exception, subsequent basic local alignment search tool (BLAST) searches confirmed that each DNA sequence had high (97%-100%) identity with *B. microti* isolate Gray.

### Statistical Analysis

Descriptive statistics were used to summarize the data. Comparisons of binomial data were made using Fisher exact tests, and continuous data were compared using Mann-Whitney tests. Data analyses were conducted using Excel (Microsoft, Redmond, Washington) and SAS Version 9.3 (SAS Institute, Cary, North Carolina).

## RESULTS

### Detection of *B microti* in Ticks

We collected 171 ticks from the region just north of La Crosse (Figure 1, Tick Collection Site 2) in 2013. By PCR, *B microti* DNA was found in 9 of 171 (5%) ticks.

### Babesiosis Diagnostic Tests

Twenty-two cases of babesiosis were diagnosed in our health system from January 1, 2004, through November 1, 2013 (Figure 2 and Table 1). Of these cases, 15 were diagnosed since the incorporation of PCR testing at our institution in the spring of 2012, including 11 cases in 2013. Prior to 2012, all laboratory diagnoses of babesiosis were based upon a peripheral smear positive for *Babesia*, which in 2 cases was confirmed by a PCR test positive for *B microti* DNA. All cases diagnosed since the tick-borne infection diagnostic panel was made available June 1, 2012, had PCR assays positive for *B microti* DNA, and only 8 of the 15 cases (53%) had a peripheral smear suggestive of babesiosis. From January 1, 2004, until June 1, 2012, 7 of 617 (1.1%) diagnostic blood smears to examine for *Anaplasma* and *Babesia* organisms were positive for *Babesia*, compared with 15 of 2521 (0.6%) PCR tests positive for *B microti* DNA after implementation of the tick-borne infection diagnostic panel. Only 8 of 2521 (0.3%) rapid smears performed in the tick-borne infection diagnostic panel were read as suspicious for *Babesia* parasites. The rapid smear was read as suspicious for *Anaplasma* organisms in 10 of 2521 (0.4%) and subsequent *Anaplasma* PCR was positive in 90 of 2521 (3.6%). All smears positive for *Babesia* or *Anaplasma* organisms were subsequently confirmed by PCR assay. Two hundred seventy-six of 2521 (10.9%) of *B burgdorferi* screens done as part of the panel were positive, and an additional 119 (4.7%) were in the indeterminate range.

### Epidemiology and Clinical Characteristics of Patients With Babesiosis

Figure 1 plots the area of residence of the patients diagnosed with babesiosis. Only 1 patient had traveled outside of the area (to northern Minnesota) in the 30 days prior to diagnosis, and none were known to have had a blood transfusion in the 6 months prior to diagnosis. Only 6 patients (27%) had documentation of a recent tick bite, although 17 patients (72%) recalled outdoor activities likely to have exposed them to ticks.

Demographic and clinical details are shown in Table 2. Only 2 patients were aged younger than 50 years, and only 1 patient had anatomic or functional asplenia. Fever was the most common presenting sign, recorded in 18 patients (82%). The median duration from symptom onset to diagnosis was 5 days (range 1-14). Erythema migrans and/or serologic evidence of co-infection with *B burgdorferi* was noted in 11 of 22 patients (50%), 1 of whom also had infection with *A phagocytophila* based upon a positive PCR.

**Table 2.** Demographic and Clinical Characteristics of the 22 Patients Diagnosed With Babesiosis in the Region Surrounding La Crosse, Wisconsin<sup>a</sup>

Demographic or Clinical Characteristic	Value
Median age, y (IQR)	68 (59-82)
Men	12 (55)
Asplenia	1 (5)
Travel outside the region	1 (5)
Known tick bite	6 (27)
Headache	11 (50)
Fatigue	18 (82)
Myalgias/artralgias	9 (41)
Nausea/vomiting	6 (27)
Erythema migrans rash	4 (18)
Hospital admission	9 (41)
Median length of stay in days (range)	6 (1-10)

<sup>a</sup>Data are presented as number (percentage) of patients unless otherwise indicated.  
Abbreviation: IQR, interquartile range.

**Table 3.** Laboratory Findings in the 22 Patients Diagnosed With Babesiosis in the Region Surrounding La Crosse, Wisconsin<sup>a</sup>

Laboratory test	Observations	Value (IQR)
Hemoglobin, g/dL	21	11.8 (9.3-13.2)
Platelet count, ×10 <sup>9</sup> /L	21	79 (64-120)
White blood cell (WBC) count, ×10 <sup>9</sup> /L	21	5.4 (4.1-6.3)
Alanine transaminase (ALT), U/L	15	61 (37-89)
Aspartate transaminase (AST), U/L	14	72 (50-103)

<sup>a</sup>To convert hemoglobin to g/L, multiply by 10; to convert platelet count to ×10<sup>3</sup>/μL, divide by 1; to convert WBC to /μL, divide by 0.001; to convert ALT or AST to μkat/L, multiply by 0.0167.  
Abbreviations: IQR, interquartile range.

Laboratory findings are summarized in Table 3. Thrombocytopenia and liver transaminase elevations were documented in 18 of 21 patients (86%) and 15 of 17 patients (88%) in whom testing was performed, respectively. Anemia, defined as a hemoglobin concentration below the laboratory reference range, was present in 13 of 21 patients (62%).

Clinical and laboratory features suggested a higher acuity of illness in the patients with diagnoses made prior to 2012. Anemia was present in 7 of 7 patients (100%) with diagnoses prior to 2012 and in 6 of 15 patients (40%) with diagnoses since the tick-borne infection diagnostic panel was implemented ( $P=.02$ ). The median hemoglobin prior to 2012 was 9.3 mg/dL, compared with 12.1 mg/dL since implementation of the tick-borne infection diagnostic panel ( $P=.04$ ). All 7 patients with diagnoses prior to 2012 had anemia, thrombocytopenia, and liver transaminase elevations. Of the 7 patients with diagnoses between 2004 and 2011, 6 required hospitalization, while only 3 of 15 with diagnoses since 2012 required hospitalization ( $P=.007$ ). The median length of hospital stay was 6 days (range 1-10 days), and no patient required intensive care unit admission.

Targeted babesiosis treatment consisted of azithromycin and

atovoquone in 18 of 21 patients, clindamycin and quinine in 1 patient, azithromycin monotherapy in 1 patient, and no treatment in 1 patient (the latter 2 patients refused atovoquone treatment due to cost concerns). In all cases, patients improved and demonstrated no ongoing symptoms or signs of infection when seen in follow-up. Both patients who refused atovoquone treatment had resolution of all symptoms 1 month following diagnosis, and a repeat PCR at that time was negative for *B microti*. Additionally, 19 patients were treated with doxycycline, and 1 patient with amoxicillin for confirmed or presumed *B burgdorferi* infection. No patients were treated with exchange transfusion.

## DISCUSSION

This study demonstrates the emergence of clinically relevant cases of babesiosis in the region surrounding La Crosse, Wisconsin, in the last decade. There were 19 confirmed cases of babesiosis in our health system from 2010 through 2013, compared with only 3 cases from 2004 through 2009, and there were as many confirmed cases in 2013 in our health system as reported in the entire state of Wisconsin in 2005.<sup>7</sup> Our results also suggest that a tick-borne infection diagnostic panel consisting of Lyme serology and *Babesia* and *Anaplasma* PCR tests is a useful diagnostic approach in patients with fevers during the tick season who reside in regions endemic for these infections. Increased babesiosis diagnoses correlated with the availability of the tick-borne infection diagnostic panel to clinicians.

Our 19-county service area is largely rural, with a population that is 93.3% white, 2.4% Hispanic, and 1% to 2% black, Asian, and other. No demographic shift has occurred to which the increase in the number of babesiosis diagnoses might be attributed. Likewise, health care delivery in our service area has not changed, with 2 large multispecialty health systems continuing to provide the vast majority of care in the region.

The rise in the number of babesiosis diagnoses is likely multifactorial. First, the geographic area of endemicity seems to have expanded. Evidence for this includes a survey of 100 ticks in our region (Figure 1, Tick Collection Site 1) in 2006 that demonstrated no evidence of *B microti*. However, we demonstrated a tick infection rate of 5% in ticks sampled in 2013 from a nearby site (Figure 1, Tick Collection Site 2). A clinical study conducted in 1997 and 1998 from a region just north of ours demonstrated no cases of babesiosis in 62 patients with a summertime systemic febrile illness seeking urgent care.<sup>8</sup> Previously, clinically relevant cases of babesiosis usually have been linked to residence or travel to northwestern Wisconsin.<sup>2,7,9</sup> In our study, we demonstrated clinically significant cases of babesiosis in patients with no travel history who resided south of La Crosse. These regions have to our knowledge heretofore not been known to be endemic for *B microti*.

Second, the availability of the tick-borne infection diagnostic

panel beginning June 1, 2012, resulted in a substantial increase in the volume of diagnostic testing specifically aimed at detecting *Babesia* infection. This likely increased babesiosis diagnoses that otherwise may have gone clinically unrecognized. It is possible that some patients diagnosed with babesiosis using our new diagnostic approach may have gone on to have resolution of their illness with no adverse outcomes, evidenced by the fact that 1 patient in our cohort was not treated and clinically did well. Further support that our testing identified less severely ill patients is that laboratory abnormalities were more marked and more patients were hospitalized prior to implementation of the tick-borne infection diagnostic panel in 2012. Conversely, and importantly, it is possible that earlier diagnosis and therapy of babesiosis with our tick-borne infection diagnostic panel may have prevented more severe illness.

Finally, the routine use of a highly sensitive and specific PCR assay for diagnosis instead of the more labor-intensive manual review of blood smears, which often requires multiple samples to be reviewed to achieve optimal sensitivity, also may have contributed to increased diagnoses. The PCR was positive for *B microti* in 7 of 15 patients (47%) who had rapid smear results negative for *Babesia* organisms. This is consistent with previously published reports that suggest that a PCR assay demonstrated enhanced diagnostic sensitivity over microscopic examination.<sup>11</sup> Additionally, *B microti* PCR is less labor intensive and can be performed on hemolyzed blood, shortcomings of microscopic examination.

Although the volume of testing and the increased sensitivity of the PCR assay may well have contributed to enhanced diagnostics, our data suggest an evolution of clinically relevant babesiosis in our region. We have newly demonstrated the presence of *B microti* in ticks in our region. Eleven patients had confirmed babesiosis in 2013—as many as in the prior 9 years combined. Hospitalizations for babesiosis in our health system increased from 3 over the years 2004 through 2009 to 6 during the years 2010 through 2013.

In our cohort, the most common abnormal laboratory test results in patients with babesiosis were liver transaminase elevation in 86% and thrombocytopenia in 88% of patients, respectively, while anemia was noted in only 62% of patients. Pfeiffer et al<sup>7</sup> demonstrated similar laboratory findings in patients with documented babesiosis. Therefore, based upon our results and those of prior studies, we conclude that *Babesia* testing in patients with systemic febrile illnesses in a region with babesiosis is best utilized in patients who are immunocompromised, over the age of 50 years, or with laboratory evidence of liver transaminase elevation or anemia. Human granulocytic anaplasmosis commonly presents with a systemic febrile illness and thrombocytopenia, leukopenia, and liver transaminase elevation. It is common practice that patients presenting with these findings

from areas endemic for *A phagocytophila* be treated for presumptive human granulocytic anaplasmosis during the appropriate season. Our study emphasizes the importance of considering babesiosis in febrile patients from endemic regions with elevated liver transaminases or thrombocytopenia because the antimicrobial treatment regimen for babesiosis differs from that for anaplasmosis.

Transfusion-associated babesiosis is a recognized risk for patients receiving blood transfusions, and it occurs most frequently in states endemic for *B microti*.<sup>12</sup> None of the patients in our study had received a blood transfusion in the 6 months prior to diagnosis of babesiosis. Nonetheless, the increase in clinically recognized cases of babesiosis in our region in recent years underscores the importance of considering transfusion-associated babesiosis in appropriate patients, particularly in the very young, old, and immunocompromised patients, in whom it may be a life-threatening infection.<sup>11</sup>

Our study has a number of limitations primarily related to its retrospective nature. We do not have baseline surveillance data of the frequency of *B microti* in ticks in our region prior to 2006 or seroprevalence data from our patient population to gauge the extent to which clinically unrecognized babesiosis may have been present. Previously published seroprevalence studies have suggested up to 10% positivity in *B microti*-endemic areas.<sup>13</sup> Additionally, we did not assess for the presence of other emerging tick-borne pathogens such as *E muris*-like agent or *Borrelia miyamotoi*, which share the same tick vector and could have been present in coinfections in our patient population. Our study also did not evaluate for the presence of another tick-borne pathogen recently identified in our region, Powassan virus. The vector for this virus is also *Ixodes* ticks. Powassan virus can cause severe encephalitis, for which there is no specific treatment.

It should be stressed that although we have demonstrated an increased number of babesiosis diagnoses in our region in recent years, results of the tick-borne infection diagnostic panel were negative for the vast majority of patients tested. Thus, empiric antimicrobial treatment ought to be used judiciously and is best reserved for patients with compelling clinical and laboratory features of a tick-borne illness.

In conclusion, we have demonstrated for the first time the presence of *B microti* in ticks and evidence of the emergence of clinically relevant cases of babesiosis in recent years in our region. Additionally, we have shown that a tick-borne infection diagnostic panel composed of Lyme serology and PCR assays for the detection of *A phagocytophilum* and *B microti* may be an effective means of enhancing diagnostic efforts. Testing for *Babesia* may be best utilized clinically if applied to immunocompromised patients, asplenic patients, patients over 50 years of age, and patients with evidence of either anemia or liver transaminase elevation.

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