Detection of *Babesia microti* and *Borrelia burgdorferi* in Host-Seeking *Ixodes scapularis* (Acari: Ixodidae) in Monmouth County, New Jersey

TERRY L. SCHULZE,¹,² ROBERT A. JORDAN,¹,³ SEAN P. HEALY,⁴,⁵ AND VIVIEN E. ROEGNER⁴,⁵

VECTOR-BORNE DISEASES, SURVEILLANCE, PREVENTION

**ABSTRACT** The etiological agents that cause human babesiosis (*Babesia microti*) and Lyme disease (*Borrelia burgdorferi*) share a common tick vector (*Ixodes scapularis* Say) and rodent reservoir (*Peromyscus leucopus*), but because the geographical distribution of babesiosis is more restricted than Lyme disease, it was not considered a nationally notifiable disease until 2011. Although recent studies have shown dramatic increases in the number of cases of babesiosis and expansion of its range, little is known about infection and coinfection prevalence of these pathogens in the primary tick vector. Of the 478 *I. scapularis* nymphs collected within six Monmouth County, NJ, municipalities between 2004 and 2006, 4.0 and 10.0% were infected with *B. microti* and *B. burgdorferi*, respectively, while 2.9% were coinfected. Analysis of the 610 *I. scapularis* adults collected during the same period yielded an infection prevalence of 8.2% for *B. microti* and 45.2% for *B. burgdorferi*, while 6.2% were coinfected. The potential public health importance of these findings is discussed.

**KEY WORDS** *I. scapularis*, *B. microti*, *B. burgdorferi*, coinfection

Human babesiosis and Lyme disease have been closely linked for nearly three decades when it was shown that their respective etiological agents, *Babesia microti* and *Borrelia burgdorferi*, share a common tick vector and rodent reservoir (*I. scapularis* Say) and *Peromyscus leucopus* (Anderson and Magnarelli 1993). Lyme disease was recognized as a potentially important public health problem and became a nationally notifiable disease in 1991 (Centers for Disease Control and Prevention [CDC] 2011b). In contrast, because the distribution of babesiosis is more restricted (Herwaldt et al. 2003, Krause et al. 2003), it did not become nationally notifiable until 2011 (CDC 2012). Although, human babesiosis has been reportable in New Jersey since 1985, the first case was not recognized until 1995 and during the 5-yr period 1995–1999, the number of confirmed cases averaged 2.6 annually (New Jersey Department of Health and Senior Services [NJDHSS] 2011). Over the last 5 yr (2006–2010), a mean of 80.4 cases have been reported each year, representing a >30-fold increase and in 2011 New Jersey ranked third in the nation with 166 reported cases (CDC 2012). A similar comparison of Lyme disease cases showed an increase of only 2.1-fold. While Lyme disease continues to be the most commonly reported tick-borne disease in the United States (CDC 2010) and New Jersey (NJDHSS 2011), 777 cases of this once rarely diagnosed disease have been reported through 2011 and human babesiosis is now considered endemic throughout New Jersey (Herwaldt et al. 2003, CDC 2012), with cases reported from each of the state’s 21 counties (NJDHSS 2011). During 2001–2008, a 20-fold increase in the number of babesiosis cases diagnosed annually was recently reported from the Lower Hudson Valley of New York (Joseph et al. 2011), confirming the contention that its range is expanding (Krause et al. 2003).

The blacklegged tick, *Ixodes scapularis* Say, is the principal vector of the causative agents of both Lyme disease and human babesiosis (Spiegelman et al. 1985). Several studies have shown that both *I. scapularis* and the Lyme disease spirochete, *B. burgdorferi*, are found throughout New Jersey (Schulze et al. 1984, 1998, 2003; Risley and Hahn 1994; Occi and Guidon 1998; Varde et al. 1998; Adelson et al. 2004). However, much less is known about the distribution of *B. microti*. Varde et al. (1996) reported a 5.0 and 43.0% infection prevalence for *B. microti* and *B. burgdorferi*, respectively, from 100 *I. scapularis* adults collected in Hunterdon County in northwestern New Jersey, while 2% were coinfected. Adelson et al. (2004) reported that 8.4 and 33.6% of 107 ticks collected in Union County in northeastern New Jersey were infected with *B.
microti and B. burgdorferi, respectively, while 1.9% were coinfected. This study reports the infection prevalence of B. microti and B. burgdorferi in I. scapularis ticks collected within Monmouth County in central New Jersey.

Materials and Methods

Study Areas. Between 2004 and 2007, we collected ticks from publicly owned land in six municipalities located in central and western Monmouth County, NJ (Fig. 1), known to consistently support large numbers of B. burgdorferi-infected I. scapularis (Dolan et al. 2009; Schulze et al. 2003, 2005a,b; 2006). The central collections were made at Naval Weapons Station (NWS) Earle, which is composed of portions of the Townships of Colts Neck, Howell, Tinton Falls, and Wall and considered a single site. The western collections were made at the adjacent Assunpink Wildlife Management Area and Perrineville Lake County Park, portions of which are located in Millstone Township and the Borough of Roosevelt, and considered a single site. All collections were made in hardwood forests within the mixed-oak woodland complex characteristic of New Jersey’s Inner Coastal Plain (Collins and Anderson 1994).

Fig. 1. Location of I. scapularis collection areas in central and western Monmouth County, NJ.

Tick Collections. Between fall 2004 and spring 2007, sites were sampled during the seasonal activity peak of either I. scapularis adults (April), nymphs (May–June), or both (Schulze et al. 1986) using a combination of walking surveys and standard drag sampling techniques (Ginsberg and Ewing 1989, Schulze et al. 1997). All ticks adhering to investigators’ coveralls and drags were placed in glass vials containing 70% ethanol and held at −80°C until analysis.

DNA Extraction. DNA was isolated from I. scapularis using a standardized extraction protocol (Schulze et al. 2003). Individual adult ticks and nymphs were placed in 1.5 ml microfuge tubes and crushed with disposable pestles in the presence of 120 μl of DNAzol Genomic DNA isolation reagent (Molecular Research Center, Inc., Cincinnati, OH), with or without the addition of Proteinase K (Ambion, Auston, TX) at a concentration of 0.1 mg/ml. The resulting lysates were heated at 95°C for 10 min. Insoluble tissue fragments were pelleted by centrifugation at 10,000 × g for 10 min at room temperature and 100 μl of the supernatant was transferred to a 1.5 ml microfuge tube for DNA isolation. DNA was precipitated by the addition of 50 μl of 100% ethanol to the supernatant. The DNA precipitate was pelleted by centrifugation at 16,000 × g for 10 min. The supernatant was decanted and the
pellet was washed twice with 50 μl of 75% ethanol, and resuspended in 35 μl of water. Tick lysate DNA was stored at 4°C until polymerase chain reaction (PCR) analysis.

**PCR Analyses.** We used primers (FLA1-5′CATATTCAAGTGCAGAGGTCTTCA3′, FLA2-5′GAGGTGCTGATCCAGGTGCTGGCTGT3′) (Invitrogen Life Technologies, San Diego, CA) defining a 390 bp inner region of the bacterial flagellin (fla) gene (Johnson et al. 1992). Recombinant Taq polymerase, 10X PCR buffer, and dNTPs were supplied by TaKaRa Biomedicals (Otsu, Shiga, Japan). PCR reactions of 50 μl contained 0.5 μM of each primer, 200 μM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.62 U of TaKaRa Taq polymerase, 10X PCR buffer, and dNTPS were supplied by TaKaRa Biomedicals (Otsu, Shiga, Japan). PCR reactions of 50 μl contained 0.5 μM of each primer, 200 μM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.62 U of TaKaRa Taq or TaqHS, and 10 μl of tick lysate DNA. Reactions were transferred to an Eppendorf Master Gradient thermal cycler (Brinkmann Instruments, Westbury, NY). The thermal cycler program consisted of an initial 1 min 94°C denaturation step followed by 30 cycles of 30 s denaturation at 94°C, 30 s of annealing at 55°C, and 30 s of extension at 72°C, and a final extension period at 72°C (5 min). The positive control for B. burgdorferi (ATCC 35210D) was purchased from American Type Culture Collection, Manassas, VA.

The PCR assay for B. microti (Persing et al. 1992) amplified a region of the ribosomal RNA small subunit gene (ss-rDNA) using the primers: Bab1, 5′CATATTCAAGTGCAGAGGTCTTCA3′, and Bab4, 5′GAGGTGCTGATCCAGGTGCTGGCTGT3′ (Invitrogen Life Technologies, San Diego, CA) defining a 238-bp amplification product. Primers were purchased from Invitrogen Life Technologies (San Diego, CA) and assay conditions were the same as those used for the B. burgdorferi PCR. Positive control B. microti DNA was extracted from an infected hamster blood stabilate (ATCC 30221) purchased from American Type Culture Collection, Manassas, VA.

**Gel Electrophoresis.** Samples were stored at 4°C until gel electrophoresis could be accomplished. Ten microliters of each PCR amplification was analyzed by gel electrophoresis in 2.5% Tris-Acetate EDTA (TAE) agarose gels. Agarose and 10X TAE running buffer were purchased from Fisher (Suwanee, GA); 100 bp DNA ladders (Promega, Madison, WI) were included in each gel for reference. Gels were stained with ethidium bromide and photographed. A sample was considered positive if the predicted DNA fragment was present in the gel.

**DNA Purification and Sequencing.** The PCR products were gel purified using a Montage DNA Gel Extraction kit (Millipore Corporation, Bedford, MA) following the manufacturer’s protocol. The purified product was sequenced on an ABI PRISM 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, CA) at the Sequencing Facility located in the Biotechnology Center for Agriculture and the Environment at Rutgers University. Sequences were read using Chromas version 2.23 (Technelysium Pty. Ltd., Helensvale, Australia), BioEdit version 7.04.1 (Ibis Therapeutics, Carlsbad, CA), and BLASTn (v. 2.2.10). Sequences were identified by comparison to the GenBank database. All B. burgdorferi and B. microti amplicons from I. scapularis were sequenced using both the appropriate forward and reverse primer to establish and confirm identity.

**Results.**

We collected a total of 610 I. scapularis adults and 478 nymphs (Table 1). Infection prevalences in adult I. scapularis were 9.30% (B. microti), 41.18% (B. burgdorferi), and 6.33% (coinfected) from the central site and 9.49% (B. microti), 44.36% (B. burgdorferi), and 7.18% (coinfected) from the western area. Because we found no statistical difference between regions ($\chi^2 = 1.04; \text{df} = 3; P = 0.79$), we pooled the data for subsequent analysis.

Overall, 4.0 and 10.0% of I. scapularis nymphs were infected with B. microti and B. burgdorferi, respectively, while 2.9% were coinfected with both pathogens. The infection prevalence for adult I. scapularis was 8.2% for B. microti and 45.2% for B. burgdorferi, while the coinfection prevalence for B. microti and B. burgdorferi was 6.2%. There was no difference in infection prevalence for either pathogen or for coinfection between years for nymphs (G-test; $\chi^2 = 5.96; \text{df} = 5$).

**Table 1. Infection prevalence for Babesia microti and Borrelia burgdorferi in Ixodes scapularis ticks collected in Monmouth County, NJ**

<table>
<thead>
<tr>
<th>Dates</th>
<th>Stage</th>
<th>No. tested</th>
<th>% (no.) infected with Babesia microti</th>
<th>% (no.) infected with Borrelia burgdorferi</th>
<th>% (no.) coinfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Nymph</td>
<td>113</td>
<td>5.3 (6)</td>
<td>14.2 (16)</td>
<td>2.7 (3)</td>
</tr>
<tr>
<td>2005</td>
<td>Nymph</td>
<td>127</td>
<td>3.9 (5)</td>
<td>11.5 (15)</td>
<td>2.4 (3)</td>
</tr>
<tr>
<td>2006</td>
<td></td>
<td>238</td>
<td>3.4 (8)</td>
<td>7.1 (17)</td>
<td>3.4 (8)</td>
</tr>
<tr>
<td>All dates</td>
<td></td>
<td>478</td>
<td>4.0 (19)</td>
<td>10.0 (48)</td>
<td>2.9 (14)</td>
</tr>
<tr>
<td>2004/2005</td>
<td>Adult</td>
<td>201</td>
<td>5.0 (11)</td>
<td>41.3 (83)</td>
<td>4.5 (9)</td>
</tr>
<tr>
<td>2005/2006</td>
<td></td>
<td>272</td>
<td>10.7 (29)</td>
<td>43.6 (119)</td>
<td>7.7 (21)</td>
</tr>
<tr>
<td>2006/2007</td>
<td></td>
<td>137</td>
<td>7.3 (10)</td>
<td>54.0 (74)</td>
<td>5.5 (8)</td>
</tr>
<tr>
<td>All dates</td>
<td></td>
<td>610</td>
<td>8.2 (50)</td>
<td>45.2 (276)</td>
<td>6.2 (38)</td>
</tr>
</tbody>
</table>
6; P > 0.05). However, there was significant variation among years for adults (χ² = 15.03; df = 6; P < 0.05). It appears that *B. microti* infections were lower in 2004/2005 than in other years, while *B. burgdorferi* infections were higher in 2006/2007 than in previous years. Coinfections were higher in 2005/2006 than in either of the other years.

**Sequences.** The nucleotide sequences from a subset of *B. burgdorferi*-positive ticks were 97–99% homologous to the published GenBank sequence for the flagellar filament 41 kDa core protein ( flaB) region of *Borrelia burgdorferi* B31, complete sequence (GenBank AE000783.1) (Fraiser et al. 1997). The nucleotide sequences from a subset of *B. microti*-positive ticks were 96–100% homologous to the published GenBank sequence for Babesia microti isolate Gray 18S ribosomal RNA gene, partial sequence (GenBank AY693840.1) (Slemenda et al. 2004).

**Discussion**

The *B. burgdorferi* infection prevalence in *I. scapularis* adults collected from Monmouth County (44.2%) fell within the range previously reported from elsewhere in New Jersey (31.9–50.3%) (Varde et al. 1998; Schulze et al. 2003, 2005b, 2006; Adelson et al. 2004). Although the *B. microti* infection prevalence in adult ticks from Monmouth County (5.2%) was comparable to that reported in Union County (8.4%) and somewhat higher than that previously reported in Hunterdon County (5.0%), the *B. microti* and *B. burgdorferi* coinfection prevalence (6.2%) was over three-fold higher than in both Hunterdon and Union Counties (Varde et al. 1998, Adelson 2004). During the 16-yr period 1995–2010, 77 human babesiosis cases (11.7 cases/100,000 population) were reported in Monmouth County, while three cases (1.2 cases/100,000 population) and 1 cases (1.6 cases/100,000 population) were reported from Union and Hunterdon Counties, respectively. Although human babesiosis has been reported from all 21 New Jersey counties (NJDHSS 2011), the four coastal counties (Atlantic, Burlington, Monmouth, and Ocean) accounted for 63.7% of all cases reported over the last 16 yr, while these counties represent only 21.4% of New Jersey’s total population. The explanation for the observed distribution of human babesiosis cases will require further study.

The results of this and other studies suggest that throughout its range, a substantial number of *I. scapularis* are likely to be infected or coinfected with *B. burgdorferi*, *B. microti*, and/or *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis (Schulze et al. 2005b, Swanson et al. 2006). These findings have implications for clinical diagnosis and disease prevention. First, simultaneous infection with two or more of tick-borne pathogens may complicate the diagnosis and treatment of Lyme disease, lead to more severe clinical progression of any of the diseases alone, and may help explain the variable manifestations and clinical responses in some patients (Krause et al. 1996, Eskow et al. 2001, Adelson et al. 2004). Coinfection of ticks also argues for an integrated management strategy that places a high priority on the actual management of *I. scapularis* populations. In addition, public health agencies should redouble efforts to inform the medical community and public of the potential exposure to multiple *I. scapularis*-transmitted pathogens throughout much of New Jersey.

**Acknowledgments**

We thank Tom Gentile, Naval Weapons Station, Earle for his continued support. We also thank the Monmouth County Board of Chosen Freeholders and the Monmouth County Mosquito Extermination Commission for continued support of the study of tick-borne diseases. The current work was supported by a Cooperative Agreement (U50/CU219564-01,02,03) between the New Jersey Department of Health and Senior Services and the Centers for Disease Control and Prevention.

**References Cited**


**Journal of Medical Entomology** Vol. 50, no. 2


 lệSUCCESSOR


SCHULZE ET AL.: *B. microti* AND *B. burgdorferi* IN *I. scapularis* 383


