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Original article

# Relatively low prevalence of *Babesia microti* and *Anaplasma phagocytophilum* in *Ixodes scapularis* ticks collected in the Lehigh Valley region of eastern Pennsylvania

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ABSTRACT

Several human pathogens are transmitted by the blacklegged tick, *Ixodes scapularis*. These include the spirochetes that cause Lyme disease (*Borrelia burgdorferi*) which is endemic to the Lehigh Valley region of eastern Pennsylvania. Emerging and currently rare tick-borne diseases have been of increasing concern in this region, including tick-borne relapsing fever (caused by *Borrelia miyamotoi*), human granulocytic anaplasmosis (caused by *Anaplasma phagocytophilum*), and human babesiosis (caused by *Babesia microti*). Real-time PCR assays and in some instances, conventional PCR followed by DNA sequencing, were used to screen 423 DNA samples that were prepared from questing adult and nymph stage *I. scapularis* ticks for infection with four tick-borne human pathogens. *B. burgdorferi* was detected in 23.2% of the sampled ticks, while *B. miyamotoi*, *B. microti* and a human variant of *A. phagocytophilum* were detected in less than 0.5% of the ticks. Our results are consistent with those expected in a region where Lyme disease is prevalent and human cases of tick-borne relapsing fever, babesiosis and human granulocytic anaplasmosis are not currently widespread. It is expected that this study will serve as a baseline for future studies of tick-borne pathogens in an area that is in close proximity to regions of high endemicity for Lyme disease, human granulocytic anaplasmosis and human babesiosis.



## Introduction

Tick-borne infectious diseases are an issue of increasing concern in the Lehigh Valley region of eastern Pennsylvania, that includes the largely contiguous cities of Allentown, Bethlehem and Easton. Interspersed within this mostly suburban environment with ~650,000 residents in three counties (Berks, Lehigh and Northampton) are a fragmented patchwork of state, county and privately-owned properties that are suitable for several enzootic pathogen cycles, especially those that involve tick interactions with the white-footed mouse, *Peromyscus leucopus* and other competent hosts. Blacklegged ticks (*Ixodes scapularis*) are endemic to this mid-Atlantic region with a humid continental climate, southwest of the Hudson Valley region of New York (Fig. 1). The Hudson Valley region has been the focus of sustained, extensive, and recent studies on the ecological dynamics of blacklegged ticks, their vertebrate hosts and the pathogens they transmit (Aliota et al., 2014; Hersh et al., 2014; Keesing et al., 2014; Allan et al., 2003; LoGiudice et al., 2003). Recently, infection rates of *I. scapularis* with several pathogens have been reported in the Hudson Valley region (Prusinski et al., 2014; Aliota et al., 2014; Hersh et al., 2014; Keesing et al., 2014). Together, these studies provide an invaluable set of benchmarks for regional investigations of Lyme disease and other emerging tick-borne illnesses.

Lyme disease, caused by the spirochete *Borrelia burgdorferi* sensu stricto, is the most common tick-borne infection in North America, with Pennsylvania having the highest number of confirmed cases of any US state (CDC MMWR, 2013). Although the number of cases reported to the Centers for Disease Control (CDC) is likely to be an underestimate of the nation's total Lyme disease burden (Kuehn, 2013), Pennsylvania accounted for approximately 16% of the cumulative national reported cases in 2013 (CDC MMWR, 2013). In a climate-based Lyme disease risk model (Diuk-Wasser et al., 2012) that incorporated field data collected from 2004–2007, the Lehigh Valley region appeared to be in a transitional area of Lyme disease risk. The low prevalence of *B. burgdorferi*-infected

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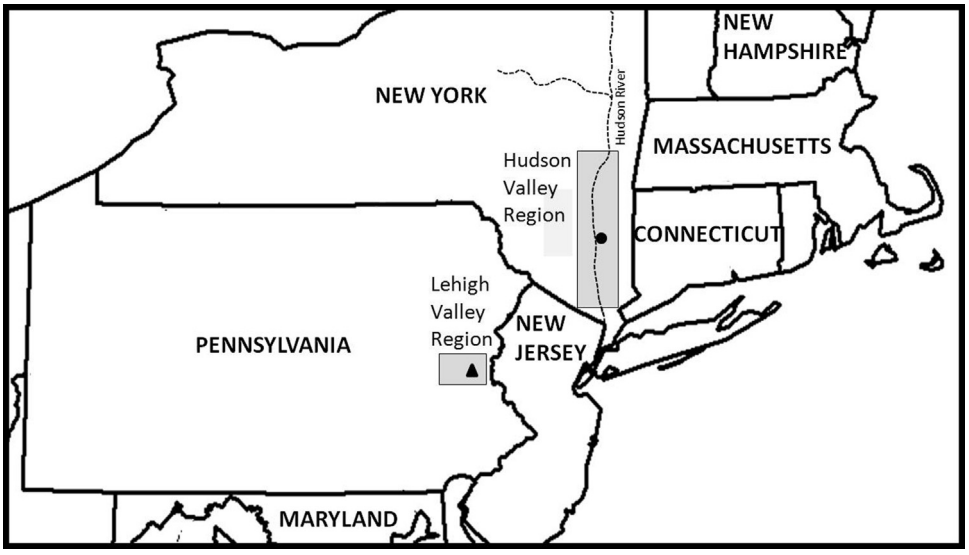


**Fig. 1.** Approximate locations of the Lehigh Valley and Hudson Valley regions. Allentown PA (▲) is separated from Poughkeepsie NY (●) by 180 km.

ticks in many regions within Pennsylvania reported in this study is not consistent with a recent study by Hutchinson et al. (2015). A change in the distribution of *B. burgdorferi*-infected ticks may be related to the widespread increases in Lyme disease cases that have been reported in the Pennsylvania County Health Profiles over the past decade (PA Dept. Heath).

*Borrelia miyamotoi*, a spirochete which causes tick-borne relapsing fever, is also transmitted by *I. scapularis* (Scoles et al., 2001). No cases of human infection with *B. miyamotoi* have been reported in Pennsylvania to date, but a severe case was reported in New Jersey, about 50 km southeast of the Lehigh Valley region (Gugliotta et al., 2013). Many commonly used PCR-based screening methods for *B. burgdorferi* cross-react with *B. miyamotoi.* As the wide North American geographic distribution of *B. miyamotoi* has been recognized (Barbour et al., 2009, Dibernardo et al., 2014), it has become increasingly important to distinguish between the two *Borrelia* species.

Human granulocytic anaplasmosis (HGA) is caused by *Anaplasma phagocytophilum*, which can be transmitted by an infective *I. scapularis* bite. These intracellular rickettsia-like bacteria preferentially invade neutrophils and infections may lead to sudden and serious influenza-like symptoms (Bakken et al., 1996). The number of HGA cases reported nationally to the CDC has increased significantly over the past decade and the spread of this pathogen northward through the Hudson Valley is discussed in (Prusinski et al., 2014). Only eight human infections with *A. phagocytophilum* in Pennsylvania were reported to the CDC in 2012 (CDC MMWR, 2012), while 34 were reported in 2013 (CDC MMWR 2013). Two strains of *A. phagocytophilum* with different host tropisms have been identified in southeastern Pennsylvania (Massung et al., 2005). One strain (AP-ha) infects humans and the other strain (Ap-Variant 1) infects white-tailed deer (*Odocoileus virginianus*) but has not yet been reported in any cases of HGA (Massung et al., 2007; Keesing et al., 2014).

*Babesia microti*, an apicomplexan protozoan parasite that is transmitted by *I. scapularis*, invades red blood cells and can cause human babesiosis, which may lead to malaria-like symptoms. Residents of the lower Hudson Valley experienced a 20-fold increase in *B. microti* infections from 2001 to 2008 (Joseph et al., 2011). Although *B. microti* infections in Pennsylvania are not yet reportable to the CDC (CDC), three cases of human babesiosis were documented in the Lehigh Valley region in 2013 (Acosta et al., 2013).

The goal of this study was to investigate the degree to which locally-acquired *I. scapularis* tick bites may pose a risk of infection with rare or emerging pathogens. Complex and interconnected environmental parameters, such as forest fragmentation (Allan et al., 2003), host biodiversity (LoGiudice et al., 2003), moisture patterns (Berger et al., 2014), and predator-prey interactions (Levi et al., 2012) have been shown to influence the community dynamics of local tick-borne pathogens. Some microbes and tick endosymbionts (Noda et al., 1997) can be detected in ticks but are not known to be transmitted to humans or cause disease. All of these factors will continue to add to the complexity of interpreting the results of regional tick-borne pathogen surveys. Annual fluctuations in the abundance of vector-borne pathogens are expected, but long-term trends that may have public health implications for more than half a million residents of the Lehigh Valley region can only be documented after baseline data have been collected.

## Methods

### *Study areas and tick collections*

Ticks were collected with permission by dragging a white fabric flag through a variety of habitats that were all associated with highly fragmented forests. Eight sites were included in this study on the basis of our ability to collect more than ten ticks at each site (Fig. 2) as per Hersh et al. (2014) and Keesing et al. (2014). Questing nymph and adults were sampled from vegetation and leaf litter under closed canopy, or adjacent clearings between April 2013 and July 2014 (Fig. 2). Most of the ticks were collected within Lehigh County. More broadly in the Lehigh Valley region, one site (Public Park near Topton) was located in Berks County, approximately 8 km from the Lehigh County border. This site was near the source of the Little Lehigh Creek, which flows into the Lehigh River. One site (Near Hackett's Park) was in Northampton County, close to the point where the Lehigh River flows into the Delaware River. An average of 53 ticks was collected from each site in the study, and the number of ticks collected ranged from 11 to 133. GPS coordinates for all of the sites are included in Supplemental Table 1 and the sites are indicated on a map (Fig. 2). We collected ticks into 70% ethanol and stored them at −20 °C until they were processed for DNA extraction. Although *I. scapularis* nymphs are the most important vectors of human disease, adults, having taken at least two