Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

A historical snapshot of *Ixodes scapularis*-borne pathogens in New Jersey ticks reflects a changing disease landscape

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A R T I C L E I N F O

Keywords:
Borrelia burgdorferi
Borrelia miyamotoi
Babesia microti
Anaplasma phagocytophilum
Hunter-killed white tailed deer
Tick-borne disease incidence
Pathogenic landscape

A B S T R A C T

Historical specimens, when available, can provide new insight into the distribution and evolution of pathogens that may not be discernible from more recent samples. We used ticks collected from hunter-killed white-tailed deer in New Jersey in 2002 to examine the prevalence and distribution of four pathogens transmitted by *Ixodes scapularis*, the blacklegged tick. Infection with *Borrelia burgdorferi* sensu stricto, *Babesia microti*, and *Anaplasma phagocytophilum* (the agents of Lyme disease, human babesiosis, and human granulocytic anaplasmosis, respectively) was highest in the Coastal Plain and lowest in the northwestern Skylands region. These patterns correspond well with the historically observed northward expansion of *I. scapularis* within New Jersey and the comparatively recent increase in human cases of these pathogens in the northern part of the state. Additionally, we provide evidence that *Borrelia miyamotoi*, a relatively new emerging pathogen and agent of relapsing fever, was widespread (though not common) throughout the state in 2002. Our findings highlight the need for enhanced awareness of tick-borne diseases other than Lyme and implementation of large-scale tick surveillance in endemic regions.

1. Introduction

Lyme disease, caused by the bacteria *Borrelia burgdorferi* sensu stricto and transmitted by the blacklegged tick, *Ixodes scapularis* Say, is the most common vector-borne disease afflicting humans in the United States. By virtue of its wide distribution and high infection prevalence in vector ticks, Lyme disease was recognized as a public health problem decades ago and became a nationally notifiable disease in 1991 (CDC, 2017). Since then both the number and geographic distribution of Lyme disease cases have continued to increase, as has the range of its main vector (Eisen et al., 2016; Mead, 2015). Most recently, expansions of *I. scapularis* and Lyme disease foci have been documented in several Midwestern states (Lantos et al., 2017; Wang et al., 2014), in the southeast (Johnson et al., 2017; Lantos et al., 2015), and in Canada (Ogden et al., 2014). While the true cause of these expansions is unknown, a number of factors have been proposed including reforestation, rebound of deer populations, and climate change (Eisen et al., 2016; Hamer et al., 2010; Ogden et al., 2010).

The recent continental expansion of *I. scapularis* and Lyme disease is reminiscent of one that occurred on a much smaller scale within New Jersey in the 1980s. Early investigations in New Jersey demonstrated that the geographic distribution of Lyme disease cases and *I. scapularis* populations were mostly coastal (Bowen et al., 1984; Schulze et al., 1984b). A 1981 survey of tick burdens on hunter-killed deer showed that *I. scapularis* was only found in southern portions of the state below the Piedmont Plain, despite deer populations remaining stable throughout the state for decades (Schulze et al., 1984b). Additionally, only 117 cases of Lyme disease were reported in New Jersey from 1978 to 1982, with all but two cases apparently contracted in coastal plain counties (Schulze et al., 1984a). Subsequently, a similar survey of ticks removed from harvested deer in 1987 showed that the range of *I. scapularis* had expanded throughout much of the northern third of the state (Schulze et al., 1998) and by the early to mid-1990s, *I. scapularis* and *B. burgdorferi* were found state-wide (Adelson et al., 2004; Goldstein et al., 1990; Orloski et al., 1998; Risley and Hahn, 1994; Schulze et al., 2003).

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https://doi.org/10.1016/j.ttbdis.2017.12.009
Received 30 March 2017; Received in revised form 10 August 2017; Accepted 11 December 2017
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With its physiographic, ecological, and demographic variation (from coastal plains to northern mountains, cedar swamps to oak forests, urban to rural development), New Jersey is a unique microcosm of the larger northeast, and a fascinating case study in the changing vector-borne disease dynamics that have afflicted this part of the country. However, due to shrinking budgets and limited resources, no statewide surveillance of tick-borne pathogens has been completed since 2000–2001. Further, as is the case nationally, the majority of tick surveillance conducted in New Jersey has targeted *B. burgdorferi,* with much less known about the distribution and rates of infection of other tick-borne pathogens (Schulze et al., 2005) even though case rates of these other illnesses have increased markedly in recent years (NJDOH, 2016).

This lesser-studied group includes other *I. scapularis*-transmitted pathogens, such as the agents of human babesiosis (*Babesia microti*) and human granulocytic anaplasmosis (*Anaplasma phagocytophilum*). In New Jersey, from 2000 to 2005 confirmed cases of babesiosis averaged 32/year, but over the last 10 years (2006–2015) a mean of 129.7 cases have been reported each year, representing a 4-fold increase (NJDOH, 2016). During that same time period anaplasmosis increased by more than 7-fold, while Lyme disease only increased by a factor of 1.4. These increases follow a pattern commonly observed in the United States where *B. burgdorferi* tends to establish first, leading to a large number of Lyme disease cases, followed by *A. phagocytophilum* and *B. microti* (and a rise in their associated illnesses) in later years (Hamer et al., 2014; Stromdahl et al., 2014). Neglecting to conduct studies of pathogens other than *B. burgdorferi* is patently unwise, as co-infection with multiple pathogens can affect transmission efficiency, produce cooperative or competitive pathogen interactions, and ultimately alter severity and complicate diagnosis of tick-borne illness in human hosts (Swanson et al., 2006).

One *I. scapularis*-borne pathogen about which we know even less, as it was only recently discovered to be pathogenic, is *Borrelia miyamotoi*, a relapsing fever spirochete first detected in *I. scapularis* ticks in the northeastern United States in 2001 (Scoles et al., 2001) and subsequently found in ticks from 15 northeastern and midwestern states (Barbour et al., 2009). The first human cases of relapsing fever attributed to *B. miyamotoi* infection were reported from central Russia (Platonov et al., 2011), with several reports of human *B. miyamotoi* infection subsequently published in the United States (Chowdri et al., 2013; Gugliotta et al., 2013; Krause et al., 2013). As with many other tick-borne diseases, case descriptions indicate that *B. miyamotoi* infection presents as a nonspecific, virus-like illness. Further, cross-reactivity during serologic testing between *B. miyamotoi* and *B. burgdorferi* may exacerbate confusion in clinical diagnosis (Krause et al., 2014). Very little is known about *B. miyamotoi* epidemiology in the United States as it is not a reportable disease.

In the absence of statewide tick surveillance efforts, an investigation of mosquito-borne infections in deer (Farajollahi et al., 2004) provided the opportunity to collect *I. scapularis* from hunter-killed animals presented at mandatory deer check stations throughout the state. White-tailed deer (*Odocoileus virginianus* L.) (hereafter “deer”) are an important source of blood for adult blacklegged ticks and several studies have suggested a relationship between deer abundance and blacklegged tick abundance (Kugeler et al., 2015). While deer are not infection reservoirs for at least three of the tick-borne human pathogens considered here (*B. burgdorferi*, *B. microti*, or *A. phagocytophilum*) (Anderson and Magnarelli, 1980; Massung et al., 2005; Piesman and Spielman, 1979; Telford et al., 1988; Walls et al., 1997), they have been proposed as important tools for the surveillance of *I. scapularis* and associated pathogens (Amerasinghe et al., 1993; Bouchard et al., 2013; Cortinas and Kitron, 2006; Magnarelli et al., 1993; Mays et al., 2014; Rosen et al., 2012; Yabsley et al., 2003). In addition, there is some speculation that deer could be a reservoir for *B. miyamotoi* (Han et al., 2016).

This paper provides a rare historical glimpse into the infection prevalence of multiple pathogens in blacklegged ticks across New Jersey, at a time shortly before a large increase in tick-borne disease cases. Unfortunately, this was also the last statewide collection of its kind, because no further collections were performed after 2002 and physical deer check stations were discontinued in 2012 and replaced with a new system allowing hunters to register their harvest either by telephone or through the Internet (NJDFW, 2016).

2. Methods

2.1. Tick collection

In 2002, during the New Jersey Six-day Firearm Season (Dec 9–14) personnel from Rutgers University and County Mosquito Control Agencies were stationed at Mandatory Deer Check stations throughout the state. In collaboration with the New Jersey Division of Fish and Wildlife and with permission of the hunters, ticks were removed from killed deer with forceps as they were checked in at the stations. Removed ticks were deposited in vials, one vial per deer, and taken to the laboratory for identification. Collected ticks were identified to species using published keys (Keirans and Clifford, 1978; Keirans and Litwak, 1989) and placed into 75% ethanol and stored in a −20 °C freezer until analysis beginning in 2013 (11 years later). Only male ticks were available for analysis, as females had been retained for other studies.

2.2. DNA extraction and analysis

DNA was isolated from the male *I. scapularis* using DNAzol® Genomic DNA isolation reagent (Molecular Research Center, Inc., Cincinnati, OH) as described in prior studies from this lab (Schulze et al., 2003). Tick lysate DNA was stored at 4 °C until PCR analysis. Lysates were tested for four *I. scapularis* pathogens: *Borrelia burgdorferi*, *Babesia microti, Anaplasma phagocytophilum,* and *Borrelia miyamotoi*, using published primers, as described below. All reactions were run with Qiagen HotStar Taq Master Mix (Qiagen Inc., Valencia, CA), according to manufacturer’s instructions, in an Eppendorf Master Gradient Thermal Cycler (Brinkmann Instruments, Inc., Westbury, NY). Reaction conditions were as follows (except as noted below): for the primary stage PCR, one cycle of 95 °C (15 min), 40 cycles of 95 °C (30 s), 55 °C (30 s) and 72 °C (1 min) and a final extension period of 72 °C (5 min). For the nested stage PCR: one cycle of 95 °C (15 min), 30 cycles of 95 °C (30 s), 55 °C (30 s) and 72 °C (1 min) and a final extension period of 72 °C (5 min).

For *B. burgdorferi*: Prior protocols amplifying a 390 bp region of the bacterial flagellin gene (using primers FLA1 and FLA2) (Johnson et al., 1992; Schulze et al., 2003) were modified by adding a semi-nested step to improve detection. The semi-nested PCR used primers FLA2 (Johnson et al., 1992) and FLALS (Barbour et al., 1996) and the same conditions described above. The positive control for *B. burgdorferi* (ATCC 35210D) was purchased from American Type Culture Collection, Manassas, VA.

For *B. miyamotoi*: A nested PCR targeting a 219 bp region of the flagellin gene was performed as described by Scoles et al. (2001) but with modified thermal cycler parameters in the nested stage: one cycle of 95 °C (15 min), 35 cycles of 94 °C (30 s), 62 °C (30 s) and 72 °C (30 s) and a final extension period of 72 °C (5 min). *B. miyamotoi* positive controls were chosen from previous *I. scapularis* samples that tested positive for *Borrelia miyamotoi* and were confirmed by sequencing.

For *B. microti*: A 238-bp region of the ribosomal RNA small subunit gene (18S rRNA) was amplified using nested PCR primers (Persing et al., 1992) as described previously (Schulze et al., 2013). These primers contain multiple 3′ mismatches to *Babesia odocoilei*, a parasite of deer. Positive control *B. microti* DNA was extracted from an infected hamster blood stabilate (ATCC 30221) purchased from American Type Culture Collection, Manassas, VA.

For *A. phagocytophilum*: Nested PCR primers were used to amplify a 546 bp region of the 16S rRNA gene (Massung et al., 1998). The *A.*

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