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Borrelia burgdorferi DNA absent, multiple *Rickettsia* spp. DNA present in ticks collected from a teaching forest in North Central Florida

Katherine Sayler[a,1], Jessica Rowland[b,c], Carisa Boyce[a,b,1], Emma Weeks[b,*]

[a] College of Veterinary Medicine, 2015 SW 16th Avenue, Veterinary Academic Building, University of Florida, Gainesville, FL, 32611, USA
[b] Department of Entomology and Nematology, PO Box 110620, 1881 Natural Area Drive, University of Florida, Gainesville, FL, 32611, USA
[c] Emerging Pathogens Institute, 2055 Mowry Rd, University of Florida, Gainesville, FL, 32611, USA

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ABSTRACT

Tick-borne diseases are an emerging public health threat in the United States. In Florida, there has been public attention directed towards the possibility of locally acquired *Borrelia burgdorferi* sensu stricto, the causative agent of Lyme disease, in association with the lone star tick. The aim of this study was to determine the prevalence of ticks and the pathogens they carry and potentially transmit, such as *B. burgdorferi*, in a highly utilized teaching and research forest in North Central Florida. Ticks were collected by dragging and flagging methods over a four month period in early 2014, identified, and tested by PCR for multiple pathogens including *Anaplasma*, *Borrelia*, *Rickettsia*, and *Ehrlichia* species. During the study period the following ticks were collected: 2506 (96.5%) *Amblyomma americanum* L., 64 (2.5%) *Ixodes scapularis* Say, 19 (0.7%) *Dermacentor variabilis* Say, and 5 (0.2%) *Ixodes affinis* Neuman. Neither *Borrelia* spp. (0/846) nor *Anaplasma* spp. (0/69; *Ixodes* spp. only) were detected by PCR in any of the ticks tested. However, *Rickettsia* DNA was present in 53.7% (86/160), 62.5% (40/64), 60.0% (3/5) and 31.6% (6/19) of *A. americanum*, *I. scapularis*, *I. affinis* and *D. variabilis*, respectively. Furthermore, *E. chaffeensis* and *E. ewingii* DNA were detected in 1.3% and 4.4% of adult *A. americanum* specimens tested, respectively. Although receiving an *A. americanum* bite is likely in wooded areas in North Central Florida due to the abundance of this tick, the risk of contracting a tick-borne pathogen in this specific area during the spring season appears to be low. The potential for pathogen prevalence to be highly variable exists, even within a single geographical site and longitudinal studies are needed to assess how tick-borne pathogen prevalence is changing over time in North Central Florida.

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1. Introduction

Tick-borne diseases are the most common vector-borne diseases in the United States. Many tick-borne diseases pose a significant public health burden in endemic areas, including *Borrelia burgdorferi*, the causative agent of Lyme disease (Bacon et al., 2008; Kuehn, 2013; Mead, 2015). In the USA, other tick-borne diseases including human monocytic ehrlichiosis and anaplasmosis, caused by *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*, respectively, are also increasing in prevalence throughout the range of their respective tick vectors (Dahlgren et al., 2011). Furthermore, tick-borne organisms responsible for human disease are being recognized at a rapid rate (Krause and Barbour, 2015; Wormser and Pritt, 2015). Upward trends in tick-borne disease prevalence and recognition are expected to continue due to increased connectivity of the world, coupled with changes in climate and land use (Khatchikian et al., 2015; Kilpatrick and Randolph, 2012), and improved diagnostic modalities.

People who work, study and visit wooded habitats, such as state parks and national forests, throughout North America are at higher risk for exposure to tick bites and their associated pathogens than those whose business and leisure activities keep them outside of these areas (Falco and Fish, 1989; Ostfeld et al., 1995). From summer 2012 to 2013, an estimated 25.6 million people visited Florida state parks, which generated $1.11 billion dollars in revenue (Park Ranger, 2015). In addition, there were just over 1000 full time employees working in Florida state parks from summer 2012 to 2013 (FDEP BNCR, 2013). Epidemiological studies have demonstrated an increased occupational risk for contracting tick-

Abbreviations: BBss, *Borrelia burgdorferi* sensu stricto; BBsl, *Borrelia burgdorferi* sensu lato.

* Corresponding author.
E-mail address: eniweeks@ufl.edu (E. Weeks).
[1] Present Address: Department of Wildlife, Ecology and Conservation, PO Box 110430, 110 Newins-Ziegler Hall, University of Florida, Gainesville, FL, 32611, USA.

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borne pathogens for park biologists and foresters (Cisak et al., 2012; Piacentino and Schwartz, 2002; Stefanoff et al., 2012; Vaughn et al., 2014; Wallace et al., 2016; Zajac et al., 2013); however, proactive surveillance of tick-borne pathogens in U.S. national parks is limited (Eisen et al., 2013).

There are many tick species in Florida, but only five that commonly bite humans, and are capable of transmitting human pathogens (Nathavitharana and Mitty, 2015; Stromdahl and Hickling, 2012); however, their abundance varies by location and season. These species are: *Amblyomma americanum* L., *Amblyomma maculatum* Koch, *Dermacentor variabilis* Say, *Ixodes scapularis* Say, and *Rhipicephalus sanguineus* Latreille. Of these species, *A. americanum* or the lone star tick is the most common in North Florida (Springer et al., 2014), and accounts for the most human tick bites in the region. This tick, which is the primary vector for *E. chaffeensis* and *E. ewingii*, also is associated with southern tick-associated rash illness (STARI) (Childs and Paddock, 2003) and a number of other microbes. STARI involves symptoms similar to Lyme disease but occurs following the bite of *A. americanum*. The causative agent of STARI has not been confirmed, but was formerly suspected to be *Borrelia lonestari* (James et al., 2001; Moore et al., 2003; Varela et al., 2004). Due to the high abundance of *A. americanum* in the Southeast and its propensity to feed on humans, there has been question and debate as to whether this tick may carry and ultimately transmit other *Borrelia* spp., including *Borrelia burgdorferi* sensu stricto (BBss) (Clark, 2004; Clark et al., 2013).

Currently, there are no well-documented cases of Lyme disease transmitted by *A. americanum* and Florida is considered a low-incidence state for Lyme disease (Forrester et al., 2015). Infectious BBss has not been propagated from *A. americanum*, although this bacterium has been propagated from rodents and *Ixodes scapularis* collected in the Southeast (Oliver et al., 1993). There are reports in the literature of PCR detection of *Borrelia* spp. in host-seeking lone star ticks (Rudenko et al., 2016), however, vector competency studies have largely failed to demonstrate the ability of *A. americanum* to transmit the pathogen (Piesman and Sinsky, 1998). Furthermore, the sylvatic cycle for the bacteria in *I. scapularis* involving the white-footed mouse and other small rodents is incompatible with transmission of the agent by *A. americanum* (Steere et al., 2005). Vector competency experiments have demonstrated that *A. americanum* is not a vector of BBss using isolates from North Carolina, Georgia and Michigan (Piesman and Happ, 1997; Sanders and Oliver, 1996). Nonetheless, detection of BBss and *B. burgdorferi* sensu lato (BBsl) in *A. americanum* in Florida has been reported by some researchers (Clark et al., 2013) with an absence of BBss being reported by other authors (Stromdahl et al., 2015) leading to much debate on the topic and the need to further corroborate findings that BBss transmission is rare in the Southeast (Mays et al., 2014; Rosen et al., 2012).

The aim of this study was to determine the prevalence of ticks and associated pathogens in a teaching forest in North Central Florida. To determine human risk of tick-borne infection in this area, ticks were collected from Austin Cary Forest (ACF) at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) and samples were analyzed for *Ehrlichia*, *Rickettsia*, *Borrelia*, and *Anaplasma* DNA.

2. Materials and methods

2.1. Field site

The UF/IFAS ACF is a 2080 acre teaching and research forest at UF that is located northeast of Gainesville, FL, USA (29.75, −82.22). Established in 1938, UF students, staff and faculty continue to use, work and conduct research in the forest (UF/IFAS SFRC, 2014). A portion of the forest is open to the public, which is used for extension, outreach and recreation. At ACF, 70% of the forest is a mixed overstory of longleaf and slash pine, with scattered cypress and hardwoods, and an understory dominated by saw palmetto and gallberry. Another 20% of the property is dominated by sandhills with an overstory of predominantly longleaf pine and an understory of wiregrass. The remaining 10% is comprised of riparian hardwoods. At least 20 instructor-led courses, each with around 25 students, utilize the forest every year and approximately 20 research studies are conducted in the forest annually. Overall there are more than 560 academic personnel making multiple visits to ACF each year.

2.2. Tick collection

Adults, nymphs and larvae of various tick species were collected using dragging and flagging methods through potential tick habitats (Rulison et al., 2013). Potential tick habitats were identified as wooded areas with leaf litter. Throughout the study, there were 16 sampling efforts between January and April 2014. This time frame was selected as our initial primary objective was to sample *I. scapularius,* which is most frequently collected throughout the winter months in Florida, and compare their prevalence to other more common species. Ticks were transported in double-sealed containers and stored live prior to identification and processing at the UF/IFAS Entomology and Nematology Department.

2.3. Tick processing and DNA extraction

The majority of ticks were identified using general taxonomic methods to species (Keirans and Litwak, 1989; Webb et al., 1990). However, due to variation in distinguishing characteristics it was not possible to identify several *Ixodes* spp. (9/69). Three samples were sent to the U.S. National Tick Collection at Georgia Southern University (Statesboro, GA, USA) for comparison to type specimens and they were confirmed to be *I. scapularis*. However, an additional sample was later tentatively identified to be *Ixodes affinis* Neuman from photographic evidence. Subsequently, the nine *Ixodes* spp. in question were identified using SYBR real-time quantitative PCR (qPCR) and DNA sequencing (methods described below). After identification, tick specimens were washed in 10% bleach and rinsed twice with molecular-grade water. Adults were separated and stored individually. Nymphs and larvae were separated into pools of 10–15 and 47, respectively. All sorted tick specimens were stored at −20 °C. DNA was extracted in an area separate from PCR setup and post-PCR processing of specimens.

Total DNA was extracted from pooled immature and individual adult specimens using Zymo Quick-gDNA™ MiniPrep kit (Zymo Research Corporation, Irvine, CA, USA). Prior to enzymatic tissue lysis, ticks were snap frozen by submersion in liquid nitrogen and homogenized with a sterile mortar and pestle. After homogenization, specimens were digested with proteinase K and DNA was extracted per manufacturer's instructions and eluted into 50 μl of pre-heated elution buffer. Samples were then frozen at −20 °C until PCR was performed.

2.4. Ixodes species PCR

A real-time PCR assay was used to differentiate between *I. affinis* and *I. scapularis* (Wright et al., 2014b). In brief, primers specific for *I. affinis* (aff_f8 and aff_r8), and *I. scapularis* (Scap_f2.2 and Scap_r2.2), were used in a single tube SYBR green assay and a melt curve analysis was used to differentiate between species (Table 1). As per Wright et al. (2014b), 1X Bio-Rad IQ™ SYBR® Green supermix (Bio-Rad, Hercules, CA, USA) was combined with 2 μl of tick DNA and 0.5 μM of each primer for a final reaction volume of 15 μl. Ampli-

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