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Widespread distribution of ticks and selected tick-borne pathogens in Kentucky (USA)

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ABSTRACT

The geographical distribution of *Ixodes scapularis* and *Amblyomma maculatum* ticks is poorly understood in Kentucky. We conducted a convenience survey of wildlife species (white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*) and black bears (*Ursus americanus*)) for ticks from October 2015 to January 2017. We detected four tick species including *Amblyomma americanum*, *Dermacentor albipictus*, *I. scapularis* and *A. maculatum*. Although the former two tick species were previously known to be widely distributed in Kentucky, we also found that *I. scapularis* and *A. maculatum* were also widespread. Because of the limited data available for pathogens from *I. scapularis* and *A. maculatum*, we tested them for *Borrelia* and *Rickettsia* spp. by polymerase chain reaction assays. Prevalence of *Borrelia burgdorferi* sensu stricto and *Rickettsia parkeri* were 11% and 3%, respectively. These data indicate that public health measures are important to prevent tick-borne diseases in Kentucky.

1. Introduction

The dynamics of tick-borne pathogen in the United States (U.S.) are ever changing as incidence rates of cases is increasing, the geographic distribution of many pathogens and their vector(s) are expanding and novel pathogens are regularly detected. Lyme disease, caused by *Borrelia burgdorferi* sensu stricto (s.s.), is a significant public health threat in the United States. In the eastern U.S., *B. burgdorferi* s.s. is transmitted by the black-legged tick *Ixodes scapularis*. County-level *I. scapularis* distribution maps indicate a wide distribution in the eastern U.S., but data are limited for several regions, including Kentucky (Dennis et al., 1998; Eisen et al., 2016). Accurate distribution data is critical because *I. scapularis* not only transmits *B. burgdorferi* s.s. but also other pathogens of medical and veterinary importance including *B. mayonii, B. miyamotoi*, *Ehrlichia muris eauclairensis*, *Anaplasma phagocytophilum,* Powassan virus, *Babesia odocoilei* and *Babesia microti* (Nelder et al., 2016).

The Gulf Coast tick (*Amblyomma maculatum*) is another species of public health and economic importance. Although historically restricted to the Gulf Coast states, recent range expansion has been documented (Paddock and Goddard, 2015). This tick transmits *Rickettsia parkeri*, causative agent of *R. parkeri* rickettsiosis (or Boutonneuse spotted fever) in humans, and can also transmit 'Panola Mountain *Ehrlichia* sp.', a cause of human and canine ehrlichiosis (Paddock and Goddard, 2015). Among domestic dogs, *A. maculatum* is also a vector of *Hepatozoon americanum* which causes American heptatozoonosis, an often severe or fatal disease (Paddock and Goddard, 2015). In addition to pathogen transmission, *A. maculatum* is important to livestock as they can cause 'gotch ear' (abscesses, edema, extensive inflammation) and increase susceptibility to myasis. Although not currently in the United States, *Ehrlichia ruminantium,* the causative agent of heartwater in ruminants, can be experimentally transmitted by *A. maculatum*.

Our primary objective of this study was to more accurately document the distribution of *I. scapularis* and *A. maculatum* in Kentucky. We also tested selected ticks for *Borrelia* spp. and *Rickettsia* spp. to determine presence and prevalence of these important pathogens.

2. Methods

Between October 2015 and January 2017, Kentucky Department of Fish and Wildlife Resources (KDFWR) personnel collected ticks from hunter-harvested white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus canadensis*) and opportunistically from car-killed cervids and black bears (*Ursus americanus*). Additional ticks removed from two field

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biologists assisting with tick collections were submitted. Sample sizes and county of origin for samples are shown in the Supplemental Figure. All ticks were collected opportunistically. Personnel of KDFWR were instructed to collect a representative number of different 'sizes and colors' of ticks to ensure we captured the diversity of tick species and stages present. All deer in Kentucky must be checked-in and hunters are required to report the county of harvest. Ticks were preserved in 95% ethanol and identified morphologically using published keys (Clifford et al., 1961; Keirans and Litwak, 1989; Keirans and Durden, 1998).

DNA was extracted from ticks using the DNEasy Blood and Tissue Kit (Qiagen, Germantown, MD) following the manufacturer's instruction. Selected *I. scapularis* (mostly unfed or only partially engorged) were tested for *Borrelia* spp. by nested PCR targeting the *fla*B gene (detects *B. burgdorferi* sensu lato and relapsing fever *Borrelia* spp.) and all *A. maculatum* (mixture of unfed and partially engorged) were screened for *Rickettsia* spp. by nested PCR targeting the 17-kDa gene as described previously (Gleim et al., 2016). The ompA gene of *Rickettsia* positives was amplified using a nested PCR as described previously (Regnery et al., 1991). Sequences were obtained and analyzed as described (Gleim et al., 2016).

3. Results

We collected a total of 2990 ticks of four species between October 2015 and January 2017 (Table 1). Because ticks were collected opportunistically, we were unable to calculate prevalence of infestation because the number of hosts examined and found to be negative was unknown. Also, accurate tick burdens are unknown as only representative samples for identification and testing were collected. Regardless, we obtained detailed county-level distribution data for the collected ticks. *Dermacentor albipictus* and *A. americanum* were detected in 47 and 20 counties, respectively (Fig. 1A). *Amblyomma maculatum* were found in 10 counties distributed across the state (Fig. 1B). We detected *I. scapularis* in 48 counties and to illustrate the current range of *I. scapularis* within Kentucky, we combined our data (41 new county records) with data from Eisen et al. (2016) (18 counties) which indicate *I. scapularis* is present in at least 59 counties (Fig. 1C). Because ticks were collected opportunistically, these county-level maps represent the minimal distributions.

Ixodes scapularis adults and *D. albipictus* (all stages) infestations had marked seasonality with most occurring in fall and winter, (Fig. 2). In contrast, all stages of *A. americanum* were detected primarily during the spring and summer months but infrequently during other periods of the year. Similarly, *A. maculatum* adults were most common in the spring and summer months, but was also detected during the fall months.

Eleven of 59 (19%) *A. maculatum* were positive for *Rickettsia* spp. with the 17-kDa screening assay. Sequences of the ompA gene indicated eight ticks (14%) were positive for *R. andeanae,* two (3%) were infected with *R. parkeri*, and one (2%) was infected with *R. amblyommatis* (Fig. 1B). Twenty-one (11%) of 197 *I. scapularis* ticks were PCR positive for *Borrelia* spp.; all were confirmed to be *B. burgdorferi* s.s. by sequence analysis. The positive ticks were detected in nine counties throughout Kentucky (Fig. 1C).

4. Discussion

Although numerous studies indicate that *I. scapularis* is widespread in the eastern U. S., there are several states that have limited reports of this tick being present, likely due to limited surveillance (Dennis et al., 1998; Eisen et al., 2016). However, in recent years *I. scapularis* has been documented in areas outside of its historical range so surveillance is critical (Eisen et al., 2016). This shortage of data impacts public health efforts. For example, a recent study that modeled the potential distribution of *I. scapularis* using various climate and habitat factors indicated that only northern Kentucky had suitable habitat (Hahn et al., 2016). However, there were previous reports of established *I. scapularis* populations being present in southern counties and the inaccuracy was proposed to be due to limited data (Hahn et al., 2016). Our data confirm *I. scapularis* occurs throughout Kentucky and is established in numerous counties where it had not been previously reported.

We detected *B. burgdorferi* s.s. in *I. scapularis* at numerous sites across Kentucky despite similar studies in Tennessee failing to detect *B. burgdorferi* s.s. in > 800 *I. scapularis* tested (Mays et al., 2014; Rosen et al., 2012). In Kentucky, the only previous study that examined *I. scapularis* (only 2 ticks tested) for *Borrelia* spp. did not detect infection (Taft et al., 2005). Historically, there have been few human Lyme disease cases in Kentucky; however, the number of human Lyme disease cases has increased in recent years from five human cases/year (0.1 incidence, cases per 100,000 population) from 2006–2012 to 14.25/year (0.3 incidence) from 2013 to 2016 (https://www.cdc.gov/lyme/stats/tables.html). In addition, data from the Companion Animal Parasite Council (CAPC, www.capcvet.org), which collects serologic testing data from domestic dogs, shows that *B. burgdorferi*-positive dogs have been detected throughout Kentucky for the past six years (Watson et al., 2017). Although the prevalence of antibodies in dogs from Kentucky was similar during this time period (1.07–1.65%), it was higher than neighboring Tennessee (0.41–0.71%) where no *B. burgdorferi*-infected ticks have been collected and where human cases incidence rates have remained ~0.1. Because of the long-term presence of positive dogs and the increasing incidence in people, a temporal-spatial analysis of combined human case data and canine infections may provide a more accurate assessment of the risk of Lyme disease in Kentucky and other states.

Most previous surveys only reported *A. maculatum* in several west-central counties of Kentucky although a recent study (Slabach et al., 2018) reported *A. maculatum* on elk in 1–4 eastern counties (exact number or county names not given). Although there have been no *R. parkeri* rickettsiosis cases in Kentucky, *R. parkeri* has been found in ~14% of *A. maculatum* (Pagac et al., 2014). A similar prevalence (12%) was noted in surveys of *A. maculatum* ticks from several other southeastern states including, Florida, Georgia, Mississippi, Oklahoma, and South Carolina, although comparison of prevalence between various

Table 1
Number of ticks collected from cervids, black bear and people in Kentucky.

Host	n	*Amblyomma americanum*			*Amblyomma maculatum*	*Ixodes scapularis*	*Dermacentor albipictus*	
		Adults	Nymphs	Larvae	Adults	Adults	Adults	Nymphs
White-tailed deer	232	346	366	126	18	794	1112	133
Elk	17				33		37	3
Black bear	3	2			4	2		
Human	2	4			4	6		