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Short communication

Risk of encountering ticks and tick-borne pathogens in a rapidly growing metropolitan area in the U.S. Great Plains

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ABSTRACT

The prevalence of tick-borne diseases has increased dramatically in many urban areas of the U.S., yet little is known about the ecology of ticks and tick-borne pathogens in relation to characteristics of North American urban and suburban landscapes. This study aimed to begin identification of the risk of encountering ticks and tick-borne pathogens within a rapidly expanding metropolitan area in the U.S. Great Plains region. Ten sites across Oklahoma City, Oklahoma were selected for tick sampling based on presence of tick habitat and level of urbanization intensity. Sampling was conducted using CO_2 traps and flagging in June, July and October 2015. A total of 552 ticks were collected from eight of the ten sampled greenspaces. The majority of ticks collected in summer were *Amblyomma americanum* (N = 534 (97.8%)), followed by *Dermacentor variabilis* (N = 10 (1.8%)) and *Amblyomma maculatum* (N = 2 (0.3%)). *Ixodes scapularis* adult females (N = 4) and nymphal *A. americanum* (N = 2) were also collected in October 2015. Tick species diversity was highest in sites with >15% of the surrounding landscape composed of undeveloped land. *Rickettsia* sp. (including *R. amblyommii* and '*Candidatus* R. andeanae'), *Ehrlichia chaffeensis* and/or *E. ewingii* were detected in tick pools from all eight sites where ticks were found. Our data suggest that the risk of encountering ticks and tick-borne pathogens exists throughout the Oklahoma City metropolitan area and that tick populations are likely influenced by urbanization intensity. Continued research is needed to clarify the full range of abiotic and biotic features of urban landscapes that influence the risk of encountering ticks and transmitting tick-borne diseases.

1. Introduction

The emergence and increased prevalence of vector-borne diseases in U.S. urban areas is a major public health concern (Bonnefoy et al., 2008; LaDeau et al., 2015). In particular, the prevalence of ticks and tick-borne diseases has increased dramatically in many U.S. urban areas (Salgo et al., 1988; Maupin et al., 1991; Jobe et al., 2007; Rydzewski et al., 2012; Blanton et al., 2014). For example, reported U.S. human cases and ticks infected with Lyme disease (caused by *Borrelia burgdorferi*), spotted fever group (SFG) rickettsiosis (caused by various *Rickettsia* spp.) and ehrlichiosis (caused by *Ehrlichia chaffeensis* and *E. ewingii*) are known to occur in urban areas (Salgo et al., 1988; Maupin et al., 1991; Blanton et al., 2014). In addition, pathogens like the *Ehrlichia muris*-like agent (Pritt et al., 2009), *Borrelia miyamotoi* (Krause et al., 2013), Heartland virus (McMullan et al., 2012), and Bourbon virus (Kosoy et al., 2015) are either emerging or just now being detected (Telford and Goethert, 2004), and these pathogens are likely to affect urban areas in the future. Thus, urban and suburban residents are being exposed, perhaps more than ever, to the risk of several tick-borne diseases in or near their own backyards. Nonetheless, few studies in the Great Plains region have investigated tick populations and tick-borne pathogen prevalence in relation to abiotic and biotic characteristics of urban landscapes.

In this regard, we hypothesize there are strong relationships between the ecology of tick-borne pathogens and urban areas in the U.S. because: (1) the abiotic conditions (e.g., moisture and temperature) and biotic conditions (e.g., local vegetation and landscape-scale patterns of greenspace) that drive tick distributions in rural areas vary predictably with varying urbanization intensity (McDonnell et al., 1997; Pickett et al., 2001; Kalnay and Cai, 2003; McKinney, 2008), (2) populations and communities of

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other arthropods, including ants, bees, and butterflies, vary predictably across urban landscapes (Vergnes et al., 2012; Casner et al., 2014; Fortel et al., 2014; Savage et al., 2014), (3) populations of vertebrates that are common hosts for ticks—including birds and mammals—vary predictably across urban landscapes (Blair, 1996; Loss et al., 2009; Thamm et al., 2009; Hamer et al., 2012a,b), and (4) relationships between ticks and characteristics of urban areas are beginning to be found in other parts of the world (Elfving et al., 2010; Hornok et al., 2013; reviewed by Rizzoli et al., 2014 and Uspensky, 2014).

A high prevalence of tick-borne disease transmission occurs in Oklahoma, including some of the highest rates of spotted fever group (SFG) rickettsiosis (Drexler et al., 2016), ehrlichiosis (Heitman et al., 2016), tularemia (*Francisella tularensis*) (CDC, 2015), and recently, one of the three fatal cases of the rare Heartland virus (OSDH, 2014) and the second U.S. case of Bourbon virus (KFOR News, 2015). Notably, historical mapping suggests increased incidence and westward expansion in Oklahoma for both SFG rickettsiosis and ehrlichiosis with Oklahoma City geographically on the edge of this increased disease risk (Noden, unpublished data). The Oklahoma City metropolitan region therefore makes an ideal field laboratory to investigate the risk of tick-borne pathogen transmission to humans and companion canines. The city is among the top-10 fastest growing metropolitan areas in the U.S. (CNN, 2014), has 7,000 acres of parkland (along with >10,000 acres in adjacent suburbs) (City of Oklahoma City, 2015), and has a climate favorable for outdoor recreation and tick activity for 10 months of the year (Talley et al., 2014).

This study aimed to begin evaluation of the risk of exposure to ticks and tick-borne pathogens across a gradient of urbanization intensity in the rapidly expanding Oklahoma City metropolitan area. Our specific objectives were to quantify tick abundance and species diversity, as well as potential risk of exposure to tick-borne pathogens, in greenspaces with differing levels of surrounding undeveloped land.

2. Methods and materials

2.1. Study site selection

Ten sites throughout the Oklahoma City metropolitan area were sampled for ticks in June, July, and October of 2015. Using Google Earth, a larger pool of 87 candidate sites were first chosen that were publicly accessible and included substantial ground- and shrub-layer vegetation and trees that provided sufficient habitat for ticks (vegetation features were determined by "ground truthing" with the Google Street View feature). Using ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, California) and land cover data from the National Land Cover Database (NLCD; Fry et al., 2011), we calculated (in 1,000 m buffers around each site) percentage of impervious surface (i.e., buildings and pavement), percentage of high density development, and percentage of undeveloped land (defined to include NLCD cover classes for forest (all types), shrub/scrub, grass/herbaceous, pasture/hay, and woody wetland). Ten sites were then chosen among the candidate sites such that each of two classes of urban development intensity were represented (as based on the percentage of surrounding undeveloped land, Class 1: $\geq$ 15% undeveloped land; Class 2: $\leq$15% undeveloped land). Due to access and other logistical issues, an equivalent number of sites in each class could not be sampled; instead, three selected sites were in Class 1 and seven were in Class 2. Once chosen, sites were visited to ensure that favorable tick habitat was present.

2.2. Tick sampling

All 10 sites were sampled for ticks once between June 23 and July 2, 2015, a period close to the end of the peak seasonal period of foraging for many tick species in our study area. All sampling occurred between 9am and 12pm (i.e., before temperatures became too hot for tick activity). At each site, six CO_2 traps were set for one hour and 1 or 2 persons also flagged during this period. Details of these sampling methods are described in Barrett et al. (2015). CO_2 traps were placed 10–50 m apart along a transect that was defined prior to site visits using Google Earth. For flagging, one or two individuals flagged vegetation along paths and in other areas heavily used by people and their dogs. Using both types of collection techniques, we were able to detect actively questing ticks. All ticks were stored in 70% EtOH and identified in the laboratory using standard keys (Keirans and Litwak, 1989; Keirans and Durden, 1998).

One additional sampling event occurred at one site (Martin Park Nature Center) in October 2015 in the same locations used for trapping and flagging during the summer. The objective of this sampling event was to detect *Ixodes scapularis* adults, which are primarily active in Oklahoma during October and November. The only difference for this sampling event was that flagging was conducted by 10 individuals for one hour each.

2.3. Pathogen testing

Pools of collected ticks were tested for *Rickettsia* spp. and *Ehrlichia chaffeensis* and *E. ewingii* DNA using modified PCR protocols (Salazar, 2015; Mitcham, 2016). Adult ticks were grouped into pools of one to five ticks, and nymphs were grouped into pools of up to 25 ticks. After washing in de-ionized water and 70% ethanol, individual adult ticks were bisected with one half used for DNA extraction and the other half stored at −80C. Nymphs were not bisected. Individual adults or pools of nymphs were heated at 80–90 °C for fifteen minutes in 2 mL vials (SARSTEDT) with 100 uL of DNAzol® Direct sample processing reagent. After heating, zirconia/silica beads (BioSpec Products) were added and the tubes were placed in a Mini-Beadbeater-16 (BioSpec Products) for three minutes. After bead-beating, resulting supernatant was collected and stored at −20 °C until DNA testing. Prior to PCR testing, pools of DNA were created from up to five adults or 25 nymphs from the extracted samples of individual adults detailed above. Pooled samples of DNA were tested for rickettsial and ehrlichial DNA by PCR using protocols developed by Dawson et al. (1996) and modified by Salazar (2015). Pooled samples of *Amblyomma americanum, Dermacentor variabilis*, and *Amblyomma maculatum* were screened by end-point PCR for the presence of *Rickettsia* spp. using the 17kd pan-specific rickettsia primers (TZ15/TZ16) (Tzianabos et al., 1989) and confirmed using the citrate synthase (gltA) primers (CS-78/CS-323) described in Labruna et al. (2004). A nested PCR assay for *Ehrlichia* spp. specific to *E. chaffeensis* or *E. ewingii* (Dawson et al., 1996) was also used to screen all pools of ticks. Fifteen *Rickettsia* positive samples and nine positive samples of each *Ehrlichia* species were chosen for sequencing using both primer sets. The positive bands were gel extracted using a PureLink™ Quick Gel Extraction Kit (Invitrogen) and then sequenced at the Oklahoma State University Core Facility. These sequences were then searched in the nucleotide BLAST database to verify the primers amplified the targets.

3. Results

A total of 552 ticks consisting of 4 species were collected from eight of the ten sites (Table 1), including from sites in both development intensity classes (Fig. 1). The majority of ticks collected during the summer were *A. americanum* (N = 534 (97.8%))

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