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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%))

Table 1
Total ticks collected by species and site and sampling technique, including for sites with ≥15% and ≤ 15% undeveloped area in the surrounding landscape (i.e., within 1,000 m of sites), across Oklahoma City, Oklahoma, USA, in June and July 2015[a].

Sites sampled	# Traps	# Flags	Total Ticks: CO_2 traps[b]	Total Ticks/Trap	Total Ticks: Flags[b]	Total Ticks/Flag	% Undeveloped
Class 1 (≥15% undeveloped area)							
Trosper Park Archery	6	2	14	2.3	41	20.5	63.80
A. americanum			12 (75%)		40 (98%)		
D. variabilis			2 (25%)		1 (2%)		
Martin Park Nature Center	6	2	394	65.7	66	33	19.92
A. americanum			388 (98.5%)		63 (95%)		
D. variabilis			2 (0.5%)		2 (3%)		
A. maculatum			–		1 (2%)		
I. scapularis (Oct)			4 (1%)		–		
Bluff Creek Park	6	2	3	0.5	9	4.5	17.30
A. americanum			3 (100%)		6 (67%)		
D. variabilis			–		3 (33%)		
Class 2 (≤ 15% undeveloped area)							
Edwards Park	6	2	1	0.2	4	2.0	10.78
A. americanum			1 (100%)		1 (25%)		
D. variabilis			–		2 (50%)		
A. maculatum			–		1 (25%)		
Lakeshore Park	6	0	0	0.0	0	0	6.72
Lake Hefner Park	6	0	3	0.5	0	0.0	6.0
A. americanum			3 (100%)		–		
Stars and Stripes Park	6	1	2	0.3	2	2.0	2.40
A. americanum			2 (100%)		2 (100%)		
Dolese Youth Park	6	2	0	0.0	1	0.5	1.63
A. americanum			–		1 (100%)		
Trinity School	6	1	9	1.5	0	0.0	0.00
Am. americanum			9 (100%)		–		
Smitty park	4	0	0	0.0	0	0	0.00

[a] Martin Park Nature Center and Bluff Creek were also sampled once in October 2015.
[b] Total ticks = All ticks collected in one park. Under each total is the breakdown of tick species collected with appropriate percentages based solely on each park collection.

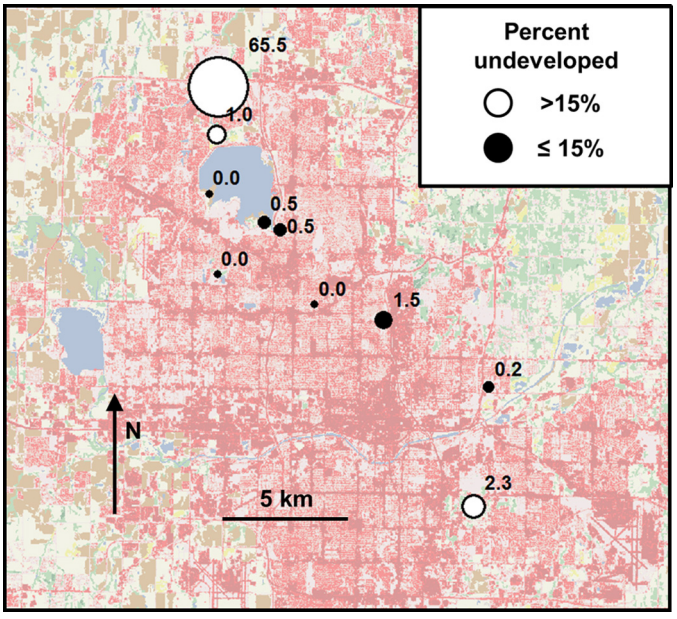

Fig. 1. Location of 10 sampling sites across Oklahoma City, Oklahoma, USA, in June and July 2015; sites were divided two classes (indicated by open and closed circles) based on the amount of undeveloped land within 1,000 m and size of site labels indicate mean number of total ticks/trap-day collected from each site (trap-days = total number of traps set at each site during the sampling period).

followed by *D. variabilis* adults (N = 10 (1.8%)) and *A. maculatum* adults (N = 2 (0.3%)). Of the 534 *A. americanum* collected, 333 (62.4%) were nymphs. *Ixodes scapularis* adult females (N = 4) and *A. americanum* nymphs (N = 2) were collected in Martin Park Nature Center during the October sampling event. Tick species diversity was highest in sites with surrounding undeveloped land greater than 15% (Table 1), and the vast majority of ticks were collected from sites with relatively high percentages of surrounding undeveloped land, including Martin Park Nature Center (N = 457 ticks; 83.7% of all ticks collected; 20% surrounding undeveloped land) and Trosper Park (N = 57 ticks; 10.1% of all ticks collected; 64% surrounding undeveloped land) (Fig. 1). However, smaller numbers of ticks were also found in highly urbanized sites with very little surrounding undeveloped land, including Trinity School (N = 9 ticks; 0% surrounding undeveloped land) and Dolese Youth Park (N = 1 tick; 2% surrounding undeveloped land).

At least one pathogen or group of pathogens (*Rickettsia* sp., *E. chaffeensis* or *E. ewingii*) was detected at each of the eight sites where ticks were collected. For *Rickettsia* spp., 54 (72%) pools of *A. americanum*, six (66.7%) pools of *D. variabilis* and one (50%) pool of *A. maculatum* tested positive (Table 2). For *E. chaffeensis*, 20 (26.7%) pools of *A. americanum* tested positive, and 14 (18.7%) pools of this species were positive for *E. ewingii*. One pool (11.1%) of *D. variabilis* tested positive for *E. chaffeensis* and one pool (11.1%) tested positive for *E. ewingii*. Of the 75 pools of *A. americanum*, fourteen (18.7%) were positive for *Rickettsia* spp. and *E. chaffeensis*, six (8.0%) were positive for *Rickettsia* spp. and *E. ewingii*, and two (2.7%) were positive for *E. chaffeensis* and *E. ewingii*. Six pools (8.0%) were positive for *Rickettsia* spp. and both *Ehrlichia* species. The only questing *A. americanum* male recovered by flagging in Dolese Youth Park (a park with 1.63% surrounding undeveloped land) was infected with *E. ewingii*. Additionally, of the four *A. americanum* ticks collected along trails in Stars and Stripes Park (a park with 2.6% surrounding undeveloped land), two were infected with *Rickettsia* spp. and one with *E. chaffeensis*.

Table 2
Tick-borne pathogens (documented using PCR analysis) in ticks sampled from 10 sites across Oklahoma City, Oklahoma, OK, in June and July 2015.

Tick species.	Stage.	Total no. ticks tested[a].	Rickettsia spp.[b]. No. positive pools/No. pools.	*E. chaffeensis*. No. positive pools/No. pools.	*E. ewingii*. No. positive pools/No. pools.
A. americanum	Adult.	199.	36/48.	15/48.	7/48.
	Nymph	332	18/27	5/27	7/27
D. variabilis	Adult	10	6/9	1/9	1/9
A. maculatum	Adult	2	1/2	0/2	0/2

[a] 6 ticks collected in Fall 2015 were not tested for pathogens.
[b] *R. amblyommii* was identified in 10 pools of *A. americanum* (7 adult and 3 nymph pools) and 4 pools of *D. variabilis* while '*Candidatus* R. andeanae' was identified in one *A. maculatum* pool consisting of a single male.

Of the fifteen positive *Rickettsia* pools that were chosen for sequencing using gltA and 17 kDa primers (samples from 5 parks included 7 pools of *A. americanum* adults, 3 pools of *A. americanum* nymphs, 4 pools of individual *D. variabilis* adults and 1 pool consisting of an individual male *A. maculatum*), all *A. americanum* (10/10) and *D. variabilis* (4/4) samples were 99–100% identical to known *Rickettsia amblyommii* sequences (Genbank sequence numbers CP012420.1 and CP003334.1). *Rickettsia*-positive *D. variabilis* samples were also 89–96% identical to *R. montanensis* (Genbank sequence no. CP003340.1). The rickettsial species in *A. maculatum* was 100% identical to known sequences of '*Candidatus* Rickettsia andeanae' (Genbank sequence nos. KT153033.1 and GU395295.1). Of the nine positive *E. chaffeensis* pools (samples from 4 parks of *A. americanum* adults (pools = 7) and nymphs (pools = 2)), all were 99–100% identical to known sequences of *E. chaffeensis* (Genbank sequence no. NR074500.1). The nine positive *E. ewingii* pools (samples from 4 parks of *A. americanum* adults (pools = 7) and nymphs (pools = 2)) were 99% identical to known sequences of *E. ewingii* (Genbank sequence no. NR044747.1).

4. Discussion

Our results demonstrate the presence of ticks across varying levels of urbanization intensity in the Oklahoma City metropolitan area, from greenspaces near the city core and surrounded by a highly urbanized landscape to parks in outer suburban areas surrounded by a relatively undeveloped landscape. Likewise, tick-borne pathogens were detected across the entire gradient of urbanization intensity: three tick species were collectively infected with at least three different pathogens of medical and veterinary importance. We also note a potential trend for increasing tick abundance in association with increasing amounts of surrounding undeveloped land. Across the United States, including in Oklahoma, the highest-risk areas for tick-borne pathogen exposure are generally considered to be outside of urban and suburban areas in grasslands, shrublands, woodlands, and agricultural areas. However, our results indicate that research in the U.S. should also address the ecology of ticks and tick-borne pathogens, and the behaviors that influence tick-borne disease risk in humans and pets, in urban and suburban areas.

Although ticks are well-studied in urban areas of Europe (Hornok et al., 2013; reviewed by Rizzoli et al., 2014 and Uspensky, 2014), very few U.S. studies have been designed with the *a priori* objective of systematically assessing tick populations and tick-borne pathogen risk in urban areas. Studies have investigated the presence of *B. burgdorferi*-infected ticks in Chicago (Jobe et al., 2007; Rydzewski et al., 2012) and New York City (Maupin et al., 1991; Daniels et al., 1997), and only two studies have quantified the risk of encountering ticks infected with non-Lyme pathogens (Salgo et al., 1988 in New York City; Blanton et al., 2014 in Little Rock, Arkansas). Two studies have evaluated tick populations in relation to urban characteristics. One study focused on several pathogens in ticks sampled from birds across an urbanization gradient in Chicago but did not explicitly assess how specific characteristics of the urban landscape were related to tick populations and risk of exposure to tick-borne pathogens (Hamer et al., 2012a, 2012b). The other study sampled *I. scapularis* in a single residential area of Westchester County, NY, and related presence of infected ticks to four broad vegetation types (woods, ecotone, ornamental vegetation, and lawns) (Maupin et al., 1991). To date, no study in the U.S. Great Plains region has systematically addressed the biotic and abiotic factors driving risk of encountering ticks and transmitting tick-borne pathogens in urban areas. Interestingly, this major geographical gap seems to exist for tick ecology research in general (Loss et al. submitted).

Notably, all but one of the 15 *Rickettsia* positive pools aligned with *R. amblyommii,* with the exception being one pool of a single *A. maculatum* that aligned with 'C.R. andeanae'. 'C. R. andeanae' has not been associated with infections in humans (Paddock et al., 2015). However, *Rickettsia amblyommii*, which is normally associated with *A. americanum* in Oklahoma (Heise et al., 2010; Barrett et al., 2014), is increasingly being recognized as a probable causal agent for a milder form of spotted fever group rickettsiosis in humans (Apperson et al., 2008; Dahlgren et al., 2016). Interestingly, all four pools of individual *D. variabilis* aligned at a higher percentage for known sequences of *R. amblyommii* than for *R. montanensis* using primers for two different genes. While this has been reported previously (Smith et al., 2010; Fritzen et al., 2011), *D. variabilis* is most typically associated with *R. rickettsii* and *R. montanensis* (Smith et al., 2010; Nadolny et al., 2014). The majority of pools that were positive for *E. chaffeensis* and *E. ewingii* were *A. americanum,* the main vector species for these *Ehrlichia* species in the U. S. (Paddock and Yabsley, 2007). Interestingly, both *Ehrlichia* species were each found in one pool consisting of an individual *D. variabilis*; while typically not associated with this tick species, these *Ehrlichia* have been previously reported in *D. variabilis* (Steiert and Gilfoy, 2002; Fritzen et al., 2011). The reasons for these somewhat unexpected pathogen/tick species associations is uncertain; however, future long-term tick studies will help clarify how prevalent these pathogens are in different tick species and whether other rickettsial species are present in the urban areas of the Great Plains.

The active host-seeking behavior displayed by the tick species collected, along with the identification of three known pathogens from 8 of the 10 urban parks sampled, suggests a high risk of tick attachment and the potential transmission of tick-borne disease for companion canines along edges of paths and manicured grass areas in urban greenspaces. While active infections with *R. amblyommii* have been reported in canines (Barrett et al., 2014; Barrett and Little, 2016), the effect of this rickettsial species on canines is unknown. The pathogenesis of *E. chaffeensis* and *E. ewingii* in canines, however, is well-established (Breitschwerdt et al., 1998; Little, 2010). Given that we found tick-borne pathogens in most urban sites sampled, including those with small numbers of ticks collected, dogs frequenting urban parks in our study region may acquire infected ticks and bring them into the home, thus posing an additional indirect health risk to humans. Thus, even sites per-

ceived by the public to have a low risk of acquiring ticks could have an associated risk of tick-borne pathogen transmission to humans or pets.

Notably, all sites with greater than 15% surrounding undeveloped land had a sampling rate of at least one adult tick per trap-day, while most sites with fewer ticks sampled were in highly urbanized landscapes with less than 15% undeveloped land. Although this pattern is preliminary and requires further investigation, the finding is suggestive that there could be predictable characteristics of urban landscapes that could be assessed in a geospatial analytical framework to enhance predictions about tick encounter risk in U.S. urban areas. While intensity of development in the landscape surrounding greenspaces might be a factor influencing tick abundance and diversity (Rizzoli et al., 2014; Uspensky, 2014; LaDeau et al., 2015; Lõhmus and Balbus, 2015); we have no data to suggest this factor affects the risk for encountering tick-borne pathogens. Indeed, ticks harboring at least one pathogen were recovered in all 8 sites where ticks were recovered. While further research into tick-host interactions is needed in urban areas, both surrounding undeveloped land and vegetation within greenspaces is likely to influence populations of white-tailed deer (*Odocoileus virginiana*), a key reservoir for many tick-borne pathogens and major transporter of ticks (Paddock and Yabsley, 2007; Walter et al., 2011). Thus, even with small tick populations, pathogen transmission cycles could persist in small fragments of undeveloped land in otherwise heavily urbanized areas.

In conclusion, we provide results of the first study of ticks and tick-borne pathogens across a major U.S. urban area in the Great Plains region. Although data are based on single sampling events at 10 sites over a two week period—factors which limit our inference due to expected variation in tick activity patterns with varying weather and environmental factors—we nonetheless demonstrate the strong likelihood that urban residents and pets are at risk of encountering multiple ticks and tick-borne pathogens in a wide variety of urban greenspaces. Based on our results suggesting possible links between urban landscape characteristics and tick abundance and diversity, we recommend further studies into the biotic and abiotic factors influencing spatiotemporal variation in tick populations in urban areas. Given the large proportion of the global and U.S. human population in urban areas, these studies will have substantial importance for future public health, medical, and veterinary efforts to prevent and treat tick-borne pathogens.

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