

Risk of Encountering Questing Ticks (Ixodidae) and the Prevalence of Tick-borne Pathogens in Oklahoma State Parks

Jessica R. Mitcham, Justin L. Talley, and Bruce H. Noden*

Department of Entomology and Plant Pathology, Oklahoma State University,
College of Agricultural Sciences and Natural Resources, Stillwater, OK 74078

Abstract. State parks, used by many for various kinds of recreation, are also places where people and their companion animals are exposed to ticks and tick-borne pathogens. While most studies in state parks in the United States have evaluated risk of encounter of Lyme-infected ticks, limited studies have focused on state parks in the Great Plains region where tick-borne rickettsial pathogens are more common. Six state parks in four ecoregions of Oklahoma were surveyed for exposure to questing ticks by flag sampling along hiking trails between April and August 2015, and pooled prevalence of tick-borne pathogens was assessed using polymerase chain reaction (PCR). Exposure to ticks varied among parks by location and month, with an encounter rate of 10.8 ticks per minute at Sequoyah State Park in eastern Oklahoma, to less than one tick per minute at Roman Nose State Park in western Oklahoma. Questing ticks were tested for *Rickettsia* sp., *Ehrlichia chaffeensis* Anderson et al. 1992 emend. Dumler et al. 2001, and *Borrelia burgdorferi* Johnson et al. 1984 emend. Baranton et al. 1992 with pooled prevalence rates between 56 and 0% differing among parks. The greatest risk of encountering ticks was at Sequoyah State Park in May 2015, with pooled prevalence rates of 93.3% (*Rickettsia* sp.) and 13.3% (*E. chaffeensis*). Results indicated that risk of exposure to ticks and tick-borne pathogens depended on the state park during summer months. These data could be used by the park system to alert visitors to risk of encountering pathogen-infected ticks in a given state park.

Introduction

Incidence of tick-borne disease is increasing in humans and companion animals in the United States (Little et al. 2014, CDC 2017) because of increased activity in recreational areas such as national and state parks, national forests, and wildlife management areas (Siikamäki 2011, Siderelis and Smith 2013). Outdoor activities (hiking, biking, walking dogs, camping) enable potential contact between visitors and their pets with pathogen-infected ticks (Eisen et al. 2013). Studies that focus on park lands in the United States are important regionally because state and national parks are normally less fragmented than surrounding areas. This is an environment in which to study how ecological and environmental factors influence the composition of tick species, together with vertebrate host/reservoir abundance in a defined habitat where human behavior often differ from risk-associated behavior in surrounding communities (Eisen et al. 2013, Johnson et al. 2017).

*Corresponding author: Email: bruce.noden@okstate.edu

While valuable for studying the ecology of ticks and tick-borne pathogens in a given region, there is a gap in the literature involving risk of ticks in park lands in the United States. Most studies during the last 30 years focused principally on *Ixodes scapularis* Say ticks and risk of acquiring *Borrelia burgdorferi*, the etiological agent of Lyme disease, as well as *B. miyamotoi*, *Anaplasma phagocytophilum*, and *Babesia microti*. Studies have included national and state parks in the northeastern and mid-Atlantic states (Falco and Fish 1989, Daniels et al. 1997, Vail and Smith 1997, Steiner et al. 2008, Han et al. 2014, Johnson et al. 2017), the north-central region of the United States (Smith et al. 1988, Gill et al. 1993, Paskewitz et al. 2001) or California (Lane 1996, Padgett and Bonilla 2011, Lane et al. 2013, Salkeld et al. 2014). While Lyme disease continues to impact the most Americans (CDC 2017), ehrlichiosis, Spotted fever rickettsiosis, tularemia, and emerging tick-borne viruses (Heartland and Bourbon) are increasing among human populations and ehrlichiosis and hepatozoonosis among companion animals in southern and central regions of the United States (CDC 2013; Little et al. 2014; Dahlgren et al. 2015, 2016; Biggs et al. 2016; Nichols Heitman et al. 2016). Pathogens have been studied in state parks in Georgia (Newhouse 1983), Tennessee (Zimmerman et al. 1988, Bloemer et al. 1990), Mississippi (Goltz et al. 2013), and Arkansas (Blanton et al. 2014).

One of the highest annual incidence rates for tick-borne diseases in the United States is in Oklahoma (Biggs et al. 2016). In the top 20 in the nation for annual visitation (ranked 15th) (Siikamäki 2011, Siderelis and Smith 2013), most park visitations in Oklahoma occurred in the summer when ticks are active (Eisen et al. 2013). The unique confluence of abundant ticks, unknown rates of infection by diseases transmitted by ticks, and high rates of recreational use make the state parks of Oklahoma ideal settings in which to investigate the ecology of tick-borne diseases and to link findings with management strategies that reduce human risk of disease. Mainly the ecology and biology of *Amblyomma americanum* (Linnaeus, 1758) were studied in state parks of Oklahoma in the 1970s and early 1980s (Semtner et al. 1971a,b; Robertson et al. 1975). Focus was on developing strategies involving vegetation management and aerial and tractor-mounted application of insecticides to eliminate the Lone star tick from large areas (Hoch et al. 1971; Mount 1981, 1984; Mount and Dunn 1983; Mount and Whitney 1984). Since the mid-1980s, nothing has been done regarding this important issue in Oklahoma state parks and no resources are available for use by the state park system to assess risk of encountering ticks and acquiring tick-borne illnesses. The purpose of this study, therefore, was to investigate levels of tick infestation and prevalence of tick borne pathogens at six state parks in different ecoregions across the state of Oklahoma during spring and summer months (April through August). These data provide valuable baseline information in Oklahoma state parks and preliminary data from which to assist the Oklahoma Department of Tourism develop targeted campaigns that substantially reduce risk of disease by park visitors.

Materials and Methods

Based on preliminary field collections in summer 2014, six state parks were chosen across the state of Oklahoma because of their representative qualities to the overall ecoregion as well as known risks for encountering various species of ticks (Fig. 1) (Woods et al. 2005). The same trails (one in each of the six state parks) were visited at the beginning of each month from April through August 2015.

To minimize effects from abiotic factors such as temperature, wind, and rain, attempts were made to sample all parks within a 5- to 7-day period at the beginning of each month. Sampling occurred at all sites except for 1 month at Lake Wister State Park because of exceptional flooding. Using methods described by Pardanani and Mather (2004), trails were sampled by flagging the vegetation (Barrett et al. 2015) along the trail in 40, 30-second segments (total flagging time = 20 minutes). Risk of encountering a questing tick in 1 hour was calculated to determine risk of encountering various species of ticks at each state park by month. Flag checking time was not included in the sampling time. Collected ticks were placed in vials of 70% ethanol then identified using established keys (Kierans and Litwak 1989, Kierans and Durden 1998) in a laboratory.

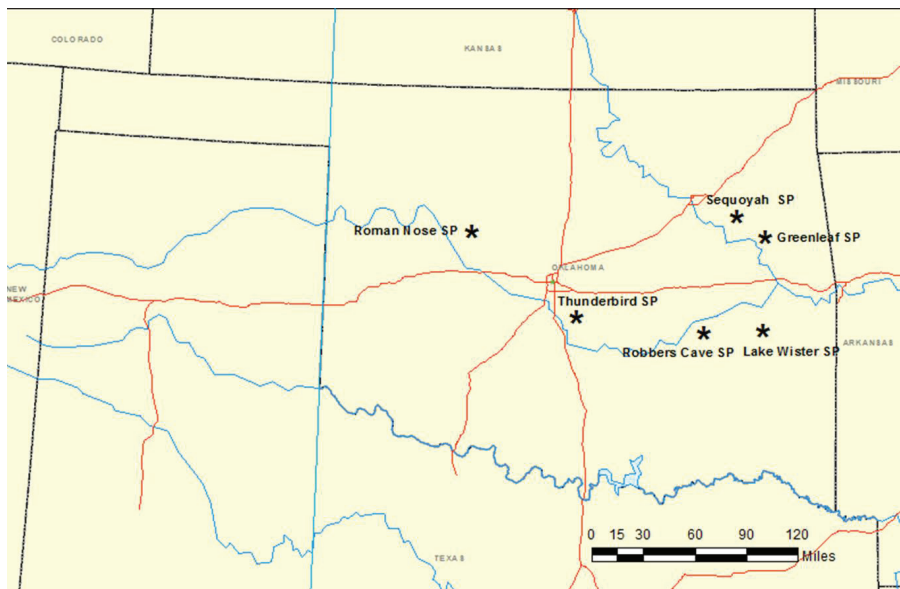


Fig. 1. Locations of six state parks surveyed for ticks in Oklahoma from April through August 2015.

Pools of collected ticks were tested for *Rickettsia* spp. and *E. chaffeensis* and *E. ewingii* DNA using modified PCR protocols (Salazar 2015, Mitcham 2016, Noden et al. 2017). Ticks were separated by collection month, state park, and individual tick species then grouped into pools of one to five ticks, and nymphs were grouped into pools of as many as 25 ticks. After washing in deionized 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 for 15 minutes at 80-90°C in 2 ml vials (Sarstedt) with 100 ul of DNAzol® Direct sample processing reagent (Molecular Research Center, Cincinnati, OH). After heating, zirconia/silica beads (BioSpec Products) were added and the tubes were placed in a Mini-Beadbeater-16 (BioSpec

Products) for 3 minutes. The resulting supernatant was collected and stored at -20°C until DNA was tested.

Before testing by PCR, pools of DNA were created from as many as five adults or 25 nymphs from the extracted samples. PCR protocols developed by Dawson et al. (1996) and modified by Salazar (2015) were used to test pooled samples of DNA for rickettsial and ehrlichial DNA. Pooled samples of *A. americanum*, *Dermacentor variabilis* (Say), and *I. scapularis* were evaluated by end-point PCR for the presence of *Rickettsia* spp. using a nested PCR assay (outer primers (R17-122/R17-500); inner primers (TZ15/TZ16) that targeted the 17-kDa protein gene (Tzianabos et al. 1989, Massung et al. 2001). A nested PCR assay (outer primers ECB/ECC and inner primers HE1/HE3) (Dawson et al. 1996) also was used to evaluate all pools of ticks for *E. chaffeensis*.

Fifteen samples positive for *Rickettsia* and 11 positive for *Ehrlichia* were randomly chosen for sequencing from the positive samples. The positive bands were extracted using a PureLink™ Quick Gel Extraction Kit (Invitrogen) and bidirectionally sequenced at the Oklahoma State University Core Facility. The sequences were searched in the nucleotide BLAST database to verify the primers amplified the targets. Pools of *I. scapularis* were also tested for *B. burgdorferi* by end-point PCR using primers ospA2/ospA4 (Scott et al. 2012). The Shannon diversity index (H) and evenness, calculated using MsExcel 2010, were used to characterize diversity of tick species at the six Oklahoma state parks.

Results

During a 5-month period, 1,035 ticks were collected at six state parks: 975 (94.2%) *A. americanum*, 37 (3.57%) *D. variabilis*, and 23 (2.22%) *I. scapularis* (Table 1). Most (72.6%) of *A. americanum* were collected at Sequoyah (51.0%) and Greenleaf (21.6%) state parks in eastern Oklahoma while 24% were collected at Lake Thunderbird State Park in central Oklahoma (Table 1, Fig. 1). Most (73.9%) *A. americanum* were collected in eastern and central Oklahoma state parks in May and June. All but two *D. variabilis* were collected at Roman Nose State Park in western Oklahoma, with most (77.1%) collected in May and July. *I. scapularis* were all collected at four state parks in eastern Oklahoma with most (82.6%) collected in April. Most *I. scapularis* (60.9%) were collected at Robbers Cave State Park.

The greatest risk for encountering ticks per minute on trails was at Sequoyah State Park, with May being the month with greatest risk (32.3 ticks per hour, potential encounter rate of 10.8 ticks per minute) followed by June (20.1 ticks per hour, potential encounter rate of 6.7 ticks per minute) (Table 1). Lake Thunderbird State Park had a similar pattern with greatest risk in May (15.6 ticks per hour, potential encounter rate of 5.2 ticks per minute) and June (10.8 ticks per hour, potential encounter rate of 3.6 ticks per minute). Encounter risk at Greenleaf State Park was low until peaking in June (18.9 ticks per hour, potential encounter rate of 6.3 ticks per minute). The least risk (less than one tick per minute) was at Lake Wister and Roman Nose state parks.

The largest Shannon H diversity and evenness values occurred at Lake Wister and Robbers Cave state parks (Table 1). This indicated greater differences in numbers of tick species and greater distribution of individual species in the community compared with Greenleaf, Lake Thunderbird, Sequoyah, and Roman Nose state parks in which one species was dominant and individual species were not distributed equally in the ecosystems sampled.

Table 1. Ticks (Adults and Nymphs) Collected, Risk of Encounter (RoE*) per Hour, Species Diversity and Evenness at Six Oklahoma State Parks from April through August, 2015

| State park | <i>A. americanum</i> (RoE) | | | | | | | | <i>D. variabilis</i> (RoE) | | | | | | | | <i>I. scapularis</i> (RoE) | | | | | | | | Shannon's | |
|------------------|----------------------------|---------------|---------------|--------------|-------------|-----|-------|-------|----------------------------|-------|-------|-------|-------|---------|---------|-------|----------------------------|-------|-------|-------|-------|-------|-------|--------|-----------|----------|
| | Apr | May | Jun | July | Aug | Ttl | Apr | May | Jun | July | Aug | Ttl | Apr | May | Jun | July | Aug | Ttl | Apr | May | Jun | July | Aug | Ttl | H | Evenness |
| Sequoyah | 46 (6.9) | 214 (32.1) | 134 (20.1) | 78 (11.7) | 25 (3.8) | 497 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.2) | 1 (0.2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 | 0.026 | 0.024 |
| Greenleaf | 9 (1.4) | 46 (6.9) | 126 (18.9) | 20 (3.0) | 10 (1.5) | 211 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5 | 0.110 | 0.100 |
| Lake Wister | 0 (0) | 1 (0.2) | 2 (0.3) | 0 (0) | 0 (0) | 3 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 | 0.673 | 0.613 |
| Robbers Cave | 3 (0.4) | 11 (1.6) | 13 (2.0) | 13 (3.0) | 13 (4.0) | 30 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 14 | 0.626 | 0.569 |
| Lake Thunderbird | 24 (3.6) | 104 (15.6) | 72 (10.8) | 32 (4.8) | 32 (4.8) | 234 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 | 0.049 | 0.044 |
| Roman Nose | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 | 0 | 0 |
| Overall total | 82 | 376 | 345 | 135 | 37 | 975 | 3 | 12 | 5 | 13 | 2 | 3 | 3 | 12 | 5 | 15 | 2 | 37 | 19 | 4 | 0 | 0 | 23 | 0.4368 | 0.397 | |

*Risk of Encounter (ticks/hour) (RoE = (number of ticks encountered per 20 minutes of flagging) X 60 minutes)) was calculated to describe risk by month of encountering questing ticks at each state park.

Of the 137 pools of *A. americanum* ticks, 97 (70.8%) were positive for *Rickettsia* sp., while two of 10 pools (20%) of *D. variabilis* were positive for *Rickettsia* sp. (Table 2). Although 43.8% of the *A. americanum* were collected at Sequoyah State Park, 93% of the pools were positive for *Rickettsia* compared with 69% of pools of *A. americanum* pools from Greenleaf State Park, 46% of pools from Lake Thunderbird State Park, and 33% of pools from Robbers Cave State Park. Only two pools of *D. variabilis* from Roman Nose State Park tested positive for *Rickettsia* sp. while no *A. americanum* from Lake Wister State Park were positive for *Rickettsia* sp. The 15 samples positive for *Rickettsia* sent for sequencing consisted of adult and nymphal *A. americanum* ticks collected at Sequoyah State

Table 2. Prevalence of Spotted Fever Group *Rickettsia* and *Ehrlichia* in Ticks (adults and nymphs combined)^a from Six Oklahoma State Parks, April to August 2015

| OK Region | State Park | Month | Tick species | # Pools | # Ticks | <i>Rickettsia</i> pools (%) | <i>Ehrlichia</i> pools (%) |
|---------------|------------------|--------|----------------------|---------|---------|-----------------------------|----------------------------|
| East | Sequoyah | April | <i>A. americanum</i> | 6 | 45 | 6 (100) | 0 |
| | | May | <i>A. americanum</i> | 28 | 211 | 25 (89.3) | 1 (4.3) |
| | | June | <i>A. americanum</i> | 16 | 134 | 15 (93.8) | 5 (31.2) |
| | | July | <i>A. americanum</i> | 8 | 78 | 8 (100) | 1 (20) |
| | | August | <i>A. americanum</i> | 2 | 25 | 2 (100) | 1 (100) |
| | | | TOTAL | 60 | 493 | 56 (93.3) | 8 (13.3) |
| East | Greenleaf | April | <i>A. americanum</i> | 2 | 8 | 2 (100) | 0 |
| | | May | <i>A. americanum</i> | 9 | 47 | 6 (66.7) | 0 |
| | | June | <i>A. americanum</i> | 17 | 126 | 11 (64.7) | 0 |
| | | July | <i>A. americanum</i> | 3 | 20 | 2 (66.7) | 0 |
| | | August | <i>A. americanum</i> | 1 | 10 | 1 (100) | 0 |
| | | | TOTAL | 32 | 211 | 22 (68.8) | 0 |
| Southeast | Lake Wister | July | <i>A. americanum</i> | 2 | 2 | 0 | 0 |
| Southeast | Robbers Cave | April | <i>A. americanum</i> | 1 | 2 | 0 | 0 |
| | | May | <i>A. americanum</i> | 2 | 11 | 2 (100) | 0 |
| | | June | <i>A. americanum</i> | 2 | 13 | 0 | 0 |
| | | July | <i>A. americanum</i> | 1 | 3 | 0 | 0 |
| | | | TOTAL | 6 | 29 | 2 (33.3) | 0 |
| South-central | Lake Thunderbird | April | <i>A. americanum</i> | 6 | 24 | 0 | 4 (80) |
| | | May | <i>A. americanum</i> | 16 | 99 | 9 (56.2) | 1 (6.7) |
| | | June | <i>A. americanum</i> | 11 | 71 | 6 (54.5) | 0 |
| | | July | <i>A. americanum</i> | 3 | 32 | 1 (33.3) | 0 |
| | | August | <i>A. americanum</i> | 1 | 2 | 1 (100) | 0 |
| | | | TOTAL | 37 | 228 | 17 (45.9) | 5 (13.5) |
| West | Roman Nose | July | <i>D. variabilis</i> | 1 | 1 | 0 | NT |
| | | April | <i>D. variabilis</i> | 1 | 3 | 1 (100) | NT |
| | | May | <i>D. variabilis</i> | 3 | 12 | 0 | NT |
| | | June | <i>D. variabilis</i> | 1 | 5 | 0 | NT |
| | | July | <i>D. variabilis</i> | 3 | 12 | 0 | NT |
| | | August | <i>D. variabilis</i> | 1 | 2 | 1 (100) | NT |
| | | | TOTAL | 9 | 34 | 2 (22.2) | |

NT = not tested

^aAdults and nymphs were combined because of small sample sizes

Park (n = 8), Greenleaf State Park (n = 2), and Lake Thunderbird State Park (n = 9), and one pool of one adult *D. variabilis* collected at Roman Nose State Park. The *Rickettsia* sp. detected in *A. americanum* collected at Sequoyah, Greenleaf, and Lake Thunderbird state parks had 98-100% homology with a known sequence of *R. amblyommatis* (previous '*Candidatus R. amblyommii*') (Accession # CP003334.1), and the *Rickettsia* spp. detected in *D. variabilis* collected from Roman Nose State Park had 99% homology with a known sequence of *R. montanensis* (Accession # CP003340.1).

Of the 137 pools of *A. americanum* tested, 13 (9.8%) were positive for *E. chaffeensis* (Table 2). Of the 13 positive pools, eight (61.5%) were from Sequoyah State Park while five (38.5%) were from Lake Thunderbird State Park. No *Ehrlichia* was found in pools of *A. americanum* collected at Greenleaf, Lake Wister, or Robbers Cave state parks. The 11 samples positive for *Ehrlichia* that were sequenced consisted of adult and nymphal *A. americanum* ticks collected at Sequoyah State Park (n = 7) and Lake Thunderbird State Park (n = 4). The *Ehrlichia* sp. detected in *A. americanum* collected at Sequoyah and Lake Thunderbird state parks had 99-100% homology with a known sequence of *E. chaffeensis* (Accession # CP007480.1). No *B. burgdorferi* was detected in 11 pools of 19 *I. scapularis* collected from four state parks.

Discussion

Principle components for developing effective tick risk avoidance strategies in recreational areas are to know when and where ticks are most active and which pathogens might be present (Eisen et al. 2013, Johnson et al. 2017). Risks of encountering ticks as well as pathogen prevalence rates were determined across six state parks in Oklahoma with each occupying specific ecological habitats that contributed to diversity and abundance of ticks in an area. Results indicated that persons recreating in parks in eastern and central Oklahoma not only needed to take precautions to avoid exposure to ticks in summer but also realize that risk assessment depends on location of the state park. Greatest risk of encountering ticks with most prevalence of *Rickettsia* sp. and *Ehrlichia* infection together with seasonal risk in May and June and the least diversity index of all state parks was at Sequoyah State Park in the central eastern region of the state. Least risk of encountering ticks with low prevalence of *Rickettsia* sp. and *Ehrlichia* infection with a relatively large species diversity index was at Robbers Cave State Park, 161 km south of Sequoyah State Park. Studies in 1978 and 1979 also reported differences in tick abundance between the two state parks (Mount 1981). Potential reasons for why tick abundance and prevalence of tick-borne pathogens differed greatly between the different parks, even those within 32 km of each other, might be related to the diverse ecoregions in which they were located.

With 12 distinct ecoregions, Oklahoma is one of the most ecologically diverse states in the United States (Woods et al. 2005). The state parks sampled were in four ecoregions, each differing slightly from the others. Although the state parks sampled were within a 161-km radius, there were definite decreases in tick abundance, risk of encounter per hour, and tick-borne pathogen prevalence, and increased species diversity from Sequoyah State Park in the central east to Robbers Cave and Lake Wister state parks in the southeastern part of the state. Sequoyah and Greenleaf state parks are in the most humid ecoregion (Boston Mountains) characterized by 16-22 cm of rainfall per year with thick oak-hickory

forests with a well-developed understory, ideal for white-tailed deer and many other animals involved in the life cycle of *A. americanum* (Semtner et al. 1971b, Woods et al. 2005). Only 50 minutes (32 km) apart in the same area of eastern Oklahoma, Sequoyah State Park is characterized by oak-hickory deciduous forest, while Greenleaf State Park is characterized mainly by prairie parkland intermingled with strips of deciduous trees such as oak-hickory that create a more temperate climate. Greatest risk of encountering ticks, as many as 11 adults per minute, and greatest prevalence rates of *Rickettsia* sp. and *Ehrlichia* sp. were at Sequoyah and Greenleaf state parks. The Greenleaf State Park trail was primarily a hiking trail that was maintained, while the Sequoyah State Park trail was used mostly as a bike trail and did not need the same amount of attention by park personnel. These differences could change the way understory and trail edges were maintained, making it more likely to encounter a tick at Sequoyah than at Greenleaf State Park. Lack of *Ehrlichia* sp. in ticks from Greenleaf State Park was surprising and needs further study to determine if there is truly a difference in prevalence at two state parks 32 km apart.

Further to the south, Lake Wister and Robbers Cave state parks are in two zones within the same ecoregion of the Arkansas Valley Plain (Woods et al. 2005). Characterized as Ouachita mixed forest with deciduous trees (maple and elm) and about 50% cover from various species of pines, the ecoregion is less humid with less ideal habitat for ticks than the Boston mountain ecoregion 80 km north. Differences in suitable habitats and the impact of different vegetation on the presence of white-tailed deer and other smaller mammals probably played a role in diversity of tick species and prevalence of tick-borne pathogens. While both state parks supported the same species of ticks in similar proportions, lack of encountering ticks at Lake Wister State Park in eastern Oklahoma, was notable. It is possible the record rains in 2015 (Lake Wister set a new rainfall record in May 2015) (NOAA 2015) impacted tick abundance. However, both sites usually were sampled within a day of each other so the data are representative. Interestingly, both parks in the region had greater diversity than other state parks. Although sampling occurred from April through August, adult *I. scapularis* that normally peak in abundance during winter months in Oklahoma were collected until May, possibly indicating that environmental conditions allowed the species to stay active longer into the spring than in other regions of the state. Both state parks in the ecoregion had much less prevalence or no detectable tick-borne pathogens compared with ticks in the northern ecoregion. Absence of *B. burgdorferi* in *I. scapularis* was not surprising because larvae of southern-clade *I. scapularis* in Oklahoma tend to feed on lizards instead of white-footed mice infected with *B. burgdorferi* unlike in the northern US (Durden et al. 2002, Garvin et al. 2015). While the low prevalence of *E. chaffeensis* was notable, it did not mean the pathogen was not present. Further study needs to confirm the finding.

While less than at Sequoyah State Park, Lake Thunderbird State Park in central Oklahoma had high sustained risk of tick encounter throughout the summer. Lake Thunderbird State Park is in the Cross-timbers ecoregion (Woods et al. 2005) consisting of oak, hickory, and eastern red cedar, the types of trees that provide excellent habitat for maintenance of *A. americanum*, in particular. Prevalence rates of both *Rickettsia* sp. and *E. chaffeensis* were comparable with ticks collected in the central east region.

In stark contrast with the other five state parks, Roman Nose State Park was characterized only by collection of *D. variabilis* during the survey. Detection of *R.*

montanensis in *D. variabilis* from Roman Nose State Park was notable because *R. montanensis* is a known human pathogen (McQuiston et al. 2012) and endosymbiont commonly associated with *D. variabilis* (Barrett et al. 2014). Located in the Central Great Plains ecoregion (Woods et al. 2005), the area is characterized by arid prairie tableland interspersed by Pleistocene sand dunes that have developed into deep gullies where vegetation can thrive, in particular rapidly expanding populations of eastern red cedar. In these gullies, small mammals and birds shelter together with white-tailed deer, providing an arid environment for development and survival of *D. variabilis*. To date, only one *A. americanum* was collected at Roman Nose State Park despite numerous sampling efforts during a 2-year period (Noden et al. 2017). While *A. americanum* was reported in the county in which Roman Nose State Park is located (Barrett et al. 2015), lack of encountering *A. americanum* on a regular basis in the park was notable and something that should be monitored in the future.

Park and recreation areas in a given state are used by residents as well as many from neighboring states as places to hike, fish, camp, and enjoy companion animals. Recreational activity, however, is normally not accompanied by an accurate assessment of risk for encountering vectors and vector-borne diseases (Bayles and Allan 2013, Eisen et al. 2013). While studies have recorded the risk of encountering *D. variabilis* and *I. scapularis* on park trails in various parts of the United States (Carroll et al. 1991, Oliver and Howard 1998, Cilek and Olson 2000, Paskewitz et al. 2001, Han et al. 2014), this Oklahoma-based study suggested high risk for encountering *A. americanum* in eastern and central state parks between April and August with relatively high risk of encountering a tick with *Rickettsia spp.* but with low risk for *E. chaffeensis*. Western state parks in Oklahoma are dominated mainly by *D. variabilis* that have relatively low prevalence of *R. montanensis*. These data can be used by state parks to promote awareness among park visitors of encountering ticks and tick-borne diseases during recreational activities. Encouraging use of personal protective measures to prevent tick bites will potentially reduce incidence of tick-borne disease in those visiting state parks (Eisen et al. 2013). This is especially important for those visiting state parks who come from areas of the state or neighboring states that might not have high rates for encountering ticks or prevalence of tick-borne diseases.

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