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# Detection of *Rickettsia amblyommatis* and *Ehrlichia chaffeensis* in *Amblyomma americanum* Inhabiting Two Urban Parks in Oklahoma

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**Abstract**

For the past 30 years, the number of people infected with causative agents of ehrlichiosis, Rocky Mountain spotted fever, and spotted fever group rickettiosis (SFGR) has increased in Oklahoma. However, there is a lack of data on pathogen prevalence within urban environments. To assess the prevalence of tick-borne pathogens in different environments, 434 *Amblyomma americanum* (lone star) ticks were collected from the environment in two parks in Edmond, Oklahoma. The presence of *Ehrlichia* spp. and spotted fever group (SFG) *Rickettsia* spp. was determined using quantitative real-time polymerase chain reaction (qPCR). 33.6% (146/434) of the *A. americanum* ticks were positive for *Rickettsia amblyommatis* and 15.2% (66/434) were positive for *Ehrlichia chaffeensis*. No ticks were positive for other SFG Rickettsiae (*R. rickettsii, R. parkeri*) or other Ehrlichiae (*E. ewingii,* and Panola Mountain *Ehrlichia*). These studies provide increased understanding of the potential risk for encountering tick-borne pathogens in urban environments.

**Keywords:** *Amblyomma americanum*, *Rickettsia amblyommatis*, *Ehrlichia chaffeensis*, Oklahoma, urban environments

Rickettsial and ehrlichial disease occurrence has been increasing among humans in the United States for the past few decades (Biggs et al. 2016). However, few studies have looked at these pathogens in urban environments or city parks (Noden et al. 2016). In Oklahoma, high rates of spotted fever group (SFG) rickettsiosis, ehrlichiosis, and tularemia occur on an annual basis in the population (Noden et al. 2016). In 2017, a total of 431 cases of Rocky Mountain spotted fever and ehrlichiosis were reported to the Oklahoma State Department of Health (OSDH 2018). Reported cases of the two diseases occurred in all months of the year (OSDH 2018).

*Amblyomma americanum* (lone star tick) is an established tick species in 68 out of 77 counties in Oklahoma (Barret et al. 2015). *A. americanum* is a pest across most of the southeastern United States, and transmits *Rickettsia* spp., *Francisella tularensis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and Heartland virus (Barrett et al. 2015). Its most common activity times are from mid-March to mid-August, and it feeds on a variety of hosts (Bouzek et al. 2013). In many studies, pathogen prevalence is determined by grouping ticks together and testing them as pools. Although testing of pooled tick samples provides evidence for the presence or absence of a pathogen in a given area, it does not accurately describe pathogen prevalence in tick populations. In this study, individual *A. americanum* ticks that were previously collected from the environment for a full year in two Edmond, Oklahoma parks (Small et al. 2019) were tested for *Rickettsia* spp. and *E. chaffeensis* by multiplex quantitative real-time polymerase chain reactions (qPCR). The two parks were in areas with different city zoning, residential (latitude 35° 41′ 8.79″ N, longitude –97° 30′ 36.37″ W) and agricultural (latitude 35° 38′ 40.20″ N, longitude –97° 21′ 45.13″ W).

Ticks were collected from June of 2016 through June of 2017 and stored in 70% ethanol at –20°C until qPCR testing. Adult and nymphal *A. americanum* ticks were dissected individually and tissues, including the midgut, were removed with a #11 scalpel blade (Varela-Stokes 2007). The tissues were placed into individual centrifuge tubes with lysis buffer and incubated at room temperature for 12–18 h (Halos et al. 2004). A GE Illustra extraction kit (GE Healthcare Life Sciences, Pittsburgh, PA) was used to extract DNA from the tissues of individual ticks. An alternative lysis buffer (NaCl 0.1 M, Tris-HCL 0.21 M, pH8 ethylenediaminetetraacetic acid [EDTA] 0.05 M, sodium dodecyl sulfate [SDS] 0.5%)

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Table 1. *Rickettsia amblyommatis* and *Ehrlichia chaffeensis* Prevalence Among *Amblyomma americanum* Ticks at a Residential and Nonresidential Park in Edmond, Oklahoma, USA as Determined by qPCR

| *Park* | *Stage (No. of ticks tested)* | *R. amblyommatis (No. of positive ticks)* | *E. chaffeensis (No. of positive ticks)* |
|---|---|---|---|
| Residential | Adults (144) | 33.3% (48) | 14.6% (21) |
| | Nymphs (145) | 36.6% (53) | 13.1% (19) |
| Nonresidential | Adults (93) | 29.0% (27) | 21.5% (20) |
| | Nymphs (52) | 34.6% (18) | 11.5% (6) |
| Total | 434 | 33.6% (146) | 15.2% (66) |

was used in place of the buffer provided by GE and the remaining extraction procedure followed the protocol provided by the manufacturer (Halos et al. 2004). Extracted DNA was stored at −20°C.

Multiplex qPCR testing was performed on a Bio-Rad CFX96 Touch Real-time PCR Detection System (Bio-Rad, Hercules, CA) and was used to detect *Ehrlichia* spp. and *Rickettsia* spp. using previously described methods and primers (Gaines et al. 2014). A 50 $\mu$L total volume reaction was used and included Bio-Rad iQ Multiplex Powermix with 10 $\mu$L of DNA. Five randomly selected positive *Rickettsia amblyommatis* samples and five randomly selected positive *E. chaffensis* samples were sequenced to confirm their identities. Primers used for sequencing were from previously published data (Kocan et al. 2000, Blair et al. 2004). Sequencing of PCR products was done by Eton Bioscience, Inc., San Diego, CA. Chi-squared tests were performed in R and used to determine statistically significant differences in bacteria prevalence within and between the two parks (R Core Team 2018). No other *Ehrlichia* or *Rickettsia* spp. were detected.

Among the 434 *A. americanum* ticks tested, *R. amblyommatis*, formerly *Rickettsia amblyommii* (Karpathy et al. 2016) was more prevalent in adults and nymphs at both sites, with a prevalence of ~33.6% (Table 1). *E. chaffeensis* was more prevalent in the adult *A. americanum* ticks at the nonresidential site. Statistical analyses showed no significant difference in bacteria prevalence between the two parks (data not shown). However, there was a significant difference in prevalence of these bacteria within each park ($p < 0.05$). The prevalence of *R. amblyommatis* was significantly higher than *E. chaffeensis* at each park. A greater prevalence of *R. amblyommatis* in *A. americanum* ticks than *E. chaffeensis* has been reported by others as well (Sanchez-Vicente et al. 2019). Whether the differences in *A. americanum* infection rates with *R. amblyommatis* and *E. chaffeensis* is biologically relevant is unclear.

Higher case rates of Rocky Mountain spotted fever (RMSF) and SFG rickettiosis (SFGR) than ehrlichiosis in Oklahoma coupled with lower prevalence of *Dermacentor variabilis* ticks, the primary vector of *Rickettsia rickettsii,* may be an indication that *R. amblyommatis* is responsible for some of the RMSF or RMSF-like illnesses reported in Oklahoma. In 2008, Apperson et al. proposed that the high *R. amblyommatis* positivity rates in *A. americanum* ticks in North Carolina may be contributing to the rise in reported RMSF cases in North Carolina (Apperson et al. 2008). Therefore, additional studies are warranted to address the possibility that *R. amblyommatis* is more than a symbiont. Of the *A. americanum* ticks tested, two were positive for both *R. amblyommatis* and *E. chaffeensis.*

The residential park is surrounded by neighborhoods and a school. It provides paved walking trails, a disc golf course, and several playgrounds. In contrast, the nonresidential park provides access to Arcadia Lake, in addition to camping spots, hiking trails, and mountain biking trails. At both sites, the two bacteria were found in both adult and nymphal *A. americanum* ticks. *Rickettsia* spp. can be transmitted transovarially and transstadially (Biggs et al. 2016), therefore, it is likely that *A. americanum* may be acting as a reservoir host and maintaining the prevalence of this potential pathogen. *Rickettsia* species were found to have a higher prevalence rate in nymphal *A. americanum* ticks tested than adults. *E. chaffeensis* is conserved by ticks transstadially, but not transovarially (Long et al. 2003). The prevalence of this pathogen in an area must be maintained by a reservoir host, specifically, the white-tailed deer (*Odocoileus virginianus*) or potentially dogs (Blanton et al. 2014).

The prevalence rate of *E. chaffeensis* was highest among *A. americanum* adults at the nonresidential site. Grund et al. (2002) found that in a park woodland area (similar to the nonresidential site in this study) within a season, the home range of white-tailed deer was larger than the home range of white-tailed deer in a residential woodland (similar to the residential park in this study) (Grund et al. 2002). Therefore, the greater prevalence of *E. chaffeensis* in *A. americanum* ticks from the nonresidential park in this study may be due to an increased presence of white-tailed deer in the nonresidential park and surrounding areas, which provides *A. americanum* ticks more opportunities to feed on infected deer.

RMSF, SFGR, and ehrlichiosis are potential life-threatening diseases among the human population (Biggs et al. 2016). As *E. chaffeenis* is an established human pathogen and *R. amblyommatis* remains a possible but unconfirmed human pathogen of SFGR or a RMSF-like disease, it is important to inform the general public of areas where these bacteria are present in the community and how to avoid their vectors. Additional studies of white-tailed deer and small mammal populations in both park types are warranted to better understand pathogen prevalence. Pathogen prevalence among other tick species such as *D. variabilis* (American dog tick), *Amblyomma maculatum* (Gulf Coast tick), and *Ixodes scapularis* (deer tick) that are present in both parks would provide more information on the potential for encountering additional tick-borne pathogens.

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### References

Apperson CS, Engber B, Nicholson WL, Mead DG, et al. Tick-borne diseases in North Carolina: Is *''Rickettsia amblyommii''* a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? Vector Borne Zoonotic Dis 2008; 8:597–606.

Barrett AW, Noden BH, Gruntmeir JM, Holland T, et al. County scale distribution of *Amblyomma americanum* (Ixodida: Ixodidae) in Oklahoma: Addressing local deficits in tick maps based on passive reporting. J Med Entomol 2015; 52:269–273.

Biggs HM, Behravesh CB, Bradley KK, Dahlgren FS, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States. MMWR Recomm Rep 2016; 65:1–44.

Blair PJ, Jiang J, Schoeler GB, Moron C, et al. Characterization of spotted fever group rickettsiae in flea and tick specimens in northern Peru. J Clin Microbiol 2004; 42:4961–4967.

Blanton LS, Walker DH, Bouyer DH. Rickettsiae and ehrlichiae within a city park: Is the urban dweller at risk? Vector Borne Zoonotic Dis 2014; 14:168–170.

Bouzek DC, Fore SA, Bevell JG, Kim HJ. A conceptual model of the *Amblyomma americanum* life cycle in Northeast Missouri. J Vector Ecol 2013; 38:74–81.

Gaines DN, Operario DJ, Stroup S, Stromdahl E, et al. *Ehrlichia* and spotted fever group *Rickettsiae* Surveillance in *Amblyomma americanum* in Virginia through use of a novel six-plex real-time PCR assay. Vector Borne Zoonotic Dis 2014; 14:307–316.

Grund MD, McAninch JB, Wiggers EB. Seasonal movements and habitat use of female white-tailed deer associated with an urban park. J Wildl Manage 2002; 66:123–130.

Halos L, Jamal T, Vial L, Maillard R, Suau A, et al. Determination of an efficient and reliable method for DNA extraction from ticks. Vet Res 2004; 35:709–713.

Karpathy SE, Slater KS, Goldsmith CS, Nicholson WL, et al. *Rickettsia amblyommatis* sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central and South America. Int J Syst Evol Microbiol 2016; 66:5236–5243.

Kocan A, Levesque GC, Whitworth LC, Murphy GL, et al. Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. Emerg Infect Dis 2000; 6:477–480.

Long SW, Zhang X, Zhang J, Ruble RP, et al. Evaluation of transovarial transmission and transmissibility of *Ehrlichia chaffeensis* (Rickettiales: Anaplasmataceae) in *ablyomma americanum* (Acari: Ixodidae). J Med Entomol 2003; 40:1000–1004.

Noden BH, Loss SR, Maichak C, Williams F. Risk of encountering ticks and tick-borne pathogens in a rapidly growing metropolitan area in the U.S. Great Plains. Ticks Tick Borne Dis 2016; 8:119–124.

Oklahoma State Department of Health Disease Statistics. 2018. https://oklahoma.gov/health/prevention-and-preparedness/acute-disease-service/publications-and-statistics.html

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2018. https://www.R-project.org/.

Sanchez-Vicente S, Tagliafierro T, Coleman JL, Benach JL, et al. Polymicrobial nature of tick-borne diseases. mBio 2019; 10:e02055-19.

Small MM, Laverty SM, King CB, Brennan RE. Tick species establishment in Oklahoma County, Oklahoma, U.S.A., identified by seasonal sampling in residential and non-residential sites. J Vector Ecol 2019; 44:105–111.

Varela-Stokes AS. Transmission of bacterial agents from lone star ticks to white-tailed deer. J Med Entomol 2007; 44:478–483.

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