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Ticks and Tick-Borne Pathogens of Residential and Non-Residential Parks in Edmond, Oklahoma

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ABSTRACT OF THESIS

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ABSTRACT:

The Oklahoma State Department of Health (OSDH) provides annual summaries of infectious diseases that occur in individuals treated in a medical facility. The summaries include five bacterial tick-borne diseases. Over the last 30 years, the number of people infected with ehrlichiosis and Rocky Mountain spotted fever has increased in Oklahoma. Few studies across the United States have sampled ticks in all months of the year and there is limited data on tick seasonality. There also is a lack of data on pathogen prevalence within urban environments. To assess the prevalence of these tick-borne pathogens in different environments across seasons, ticks were collected from two parks, one residential and one non-residential, in Edmond, Oklahoma, for one continuous year. The presence of tick-borne pathogens was determined using quantitative real-time polymerase chain reaction (qPCR). Over the year, 2,450 ticks were collected with four different species represented. Prior to this study only one of the four species collected was considered to have established populations in Oklahoma County. This study provided novel data of established populations for *Amblyomma maculatum* (Gulf Coast),

Dermacentor variabilis (American dog), and *Ixodes scapularis* (deer). Of the ticks collected, more than 90% were *Amblyomma americanum* (lone star); therefore, this species was the focus for determining pathogen prevalence. *Rickettsia rickettsii* was not detected in the ticks tested. From the residential park, 289 ticks were tested and 14% were positive for *Ehrlichia chaffeensis* and 27% for *Rickettsia amblyommii.* In contrast, *E. chaffeensis* was detected in 20% and *R. amblyommii* in 29% of 145 *A. americanum* from the non-residential park. This data can be used by the City of Edmond Parks and Recreation department to inform park visitors and caretakers of the potential risk of encountering ticks and tick-borne pathogens. Future studies are needed to determine pathogen prevalence among other tick species, additional pathogens, and host studies because the scope of my study was focused on one tick species and three bacterial pathogens. This study and future studies will provide increased understanding of the potential risk for encountering tick-borne pathogens, in addition to changing host populations, in urban environments.

CHAPTER 1

LITERATURE REVIEW

Ticks in the United States

Ticks are ectoparasitic organisms responsible for the majority of vector-borne disease transmission in the United States (Fritzen et al. 2011). Through their bite, ticks obtain and transmit pathogens, cause tick-borne paralysis, and influence potential allergic reactions (Goddard et al. 2003, van Nunen 2015). Two families of ticks are in the U.S., Argasidae (soft ticks) and Ixodidae (hard ticks), and both transmit pathogens (Parola & Raoult 2001). However, the two families differ in their feeding requirements, environments, and anatomy (Parola & Raoult 2001, Jongejan & Uilenberg 2004). Alhough both families have stages of larvae, nymphs, and adults, soft ticks differ by having multiple nymphal stages, or instars (Vial 2009). Soft ticks also feed often and rapidly in their post-larval stages and therefore, spend less time on their hosts than hard ticks (Keirans & Durden 2001). Soft ticks live near their host's nests or burrows and mate off of the host (Jongejan & Uilenberg 2004). In contrast, hard ticks often feed for long periods of time, live in the open environment, and mate while on their host (Parola & Raoult 2001, Jongejan & Uilenberg 2004). Soft ticks most commonly are found in tropical and subtropical zones because they survive well in high temperatures (some species up to 75^{o}C) and relative humidities as low as 8% (Vial 2009).

The life cycle of an Ixodidae species can take up to two years in natural habitats; however, under laboratory conditions, the life cycle has been completed in 23-32 weeks, depending on the species (Troughton & Levin 2007). The life cycle consists of three

stages (larva, nymph, and adult) impacted by host availability, habitat, and abiotic conditions (Awerbuch & Sandberg 1995). To complete their life cycle, most Ixodid ticks feed on three different hosts and for long periods of time (Parola & Raoult 2001). They feed on various host species, including humans, although humans often are considered accidental hosts (a & Lindsay 2016). Larvae and nymphs feed on small to medium sized hosts, however, adults feed on larger host species (Oliver 1989). Currently, it is unknown as to whether or not ticks have a host preference. Sonenshine and Roe (2014, p. 7) suggest that host selection may be based on how high ticks ascend vegetation when questing. Earlier life stages remain lower on the vegetation to have easier access to leaf litter and a source for humidity at the ground (Sonenshine & Roe 2014, p. 7).

Relative humidity and temperature influence tick survival and developmental time, however, few studies have provided suitable ranges. Koch (1984) found that ticks in cooler habitats (bottomland forest) required longer developmental times when compared to warmer habitats (meadows). Ticks have relative humidity requirements to avoid desiccation in the environment; these can be met by rainfall and dense leaf litter (Semtner 1971, Needham & Teel 1991). Tick survival rates are impacted by abiotic conditions of previous years (Koch 1984). In Oklahoma, Koch (1984) found that *Amblyomma americanum* (lone star tick) adults had survival rates above 90% at temperatures between 0^{o}C to 30^{o}C, and relative humidity of 65% to 85%. Nymphs had survival rates of 90% at temperatures of 12^{o}C to 30^{o}C, and relative humidity of 65 to 85%. When temperatures were below 10^{o}C, nymph survival rates decreased to 59%. These results occurred during 1978, when precipitation was below average (81.7 cm; Koch 1984). In addition, temperature affects the duration of the life cycle and cooler temperatures cause a longer

life cycle (Koch 1984, Eisen et al. 2016). Nymphs have a decreased survival rate in winter months, therefore, a prolonged life cycle is related to a decrease in nymph survival rates (Eisen et al. 2016). Different tick species have differing temperature and humidity requirements, causing geographic distribution of ticks to be determined by climate (Parola & Raoult 2001).

Nieto et al. (2018) received Ixodid tick species from citizens in 49 states (excluding Alaska) and Puerto Rico (Table 1). Species received included *A. americanum, A. maculatum* (Gulf Coast), *Dermacentor andersoni* (Rocky Mountain wood), *D. occidentalis* (Pacific Coast), *D. variabilis* (American dog), *Ixodes pacificus* (Western black-legged), and *I. scapularis* (deer). Six of these species carry and transmit human pathogens in the U.S. (Nieto et al. 2018, CDC 2018 https://www.cdc.gov/ticks/geographic_distribution.html).The Centers for Disease Control and Prevention (CDC) also provide tick distribution maps for the contiguous U.S. and Nieto et al. (2018) provides an extension of that previous data. Tick dispersal also is influenced by host migratory behavior; therefore, expansion of tick species can occur (Ogden et al. 2013). For instance, in the northeastern United States, *I. scapularis* populations expanded into southeastern Canada due to changes in land-use, climate, and tick host populations (Ogden et al. 2013). Tick species expansion also has been attributed to expansion or increase of plant populations (Noden & Dubie 2017).

In recent years, there has been little research on the prevalence of human pathogens among ticks in Oklahoma. However, tick pathogen prevalence studies in other states, such as Texas, Virginia, Georgia, Ohio, and Mississippi, have collected ticks from

pets, agricultural animals, humans, and the environment (Murphy et al. 1998, Trout et al. 2010, Williamson et al. 2010, Fitak et al. 2014, Gaines et al. 2014, Gleim et al. 2016).

Several studies have examined pathogen prevalence based on tick submissions by the general public. A study in Texas obtained ticks attached to humans and found 0.8% of *A. americanum* adult ticks were positive for *Ehrlichia spp.* and 21.3% for *Rickettsia spp.* (Williamson et al. 2010). A similar study in Georgia found 0.4% of *A. americanum* adults to have *E. chaffeensis* and 32% to have *Rickettsia spp.* (Gleim et al. 2016). Other studies have determined pathogen prevalence from ticks on differing host species. For example, a study in Kentucky observed individual tick pathogen prevalence and found rates of 28% for *Rickettsia spp.* and 6% for *E. chaffeensis* among *A. americanum* ticks (Fritzen et al. 2011). Ticks also are obtained from the environment and tested for pathogen prevalence. Gaines et al. (2014) found an average overall prevalence of 7.3% for *E. chaffeensis* and 72.8% for *R. amblyommii* across the state of Virginia for 206 *A. americanum* adult ticks. However, differing regions had prevalence as high as 24.5% for *E. chaffeensis* and 81.6% for *R. amblyommii* (Gaines et al. 2014).

The geographic location of tick-borne pathogens is dependent upon the location of the transmitting tick species, in addition to the presence of a reservoir host (Pfäffle et al. 2013). In the United States, there are 16 known tick-borne pathogens consisting of bacterial, viral, and protozoan organisms, responsible for causing minor to chronic disease and death among humans (Biggs et al. 2016, CDC 2017 https://www.cdc.gov/ticks/diseases/index.html). Ticks obtain pathogens two ways; by feeding on an infected host or by transovarial transmission, although few pathogen species, *Rickettsia spp.* and *F. tularensis,* are obtained this way (Goethert & Telford

2009, Killmaster et al. 2014). In animal models, most pathogens are transmitted from the tick to a host between 10 and 48 hours after attachment; thus, a tick could carry a pathogen or pathogens, but not feed on the host long enough for transmission to occur (Saraiva et al. 2014, Cook 2015). Therefore, basing pathogen prevalence on occurrence of sick individuals bit by ticks is not an accurate representation of true pathogen prevalence among tick populations. Approximately 50,000 people in 2016 were reported to the CDC for becoming ill with a tick-borne disease in the U.S. (CDC 2016 https://wonder.cdc.gov/nndss/static/2016/annual/2016-table1.html). This number only includes seven of the 16 potential pathogens (CDC 2016 https://wonder.cdc.gov/nndss/static/2016/annual/2016-table1.html). It also does not account for non-reported cases or misdiagnoses. Therefore, it is probable that this number underrepresents the actual number of tick-borne disease occurrence on an annual basis.

Ticks in Oklahoma

Two species of Argasidae are present in Oklahoma, however, none were collected in the preliminary study. Therefore, this study focuses on five members of Ixodidae known to carry human pathogens in central Oklahoma. According to the CDC, (https://www.cdc.gov/ticks/geographic_distribution.html) *A. americanum, A. maculatum, D. variablilis, I. scapularis*, and *Rhipicephalus sanguineus* (brown dog tick) are in Oklahoma and they must feed on one to three hosts to complete their lifecycle. A tick population is considered established if six or more ticks or more than one life stage are reported in a single collection period (Dennis et al. 1998). *Amblyomma americanum* have established populations in 68/77 counties of Oklahoma (Barrett et al. 2015). In addition,

A. maculatum is established in 50/77, *D. variabilis* in 68/77, and *I. scapularis* in 51/77 counties of Oklahoma (Mitcham et al. 2017). Recent studies have sampled ticks from state parks, city parks, and horses to determine tick populations and pathogen prevalence in Oklahoma (Duell et al. 2013, Noden et al. 2016, Mitcham et al. 2018). In each of these studies, *A. americanum* ticks accounted for over 90% of the ticks collected, even though collection methods were different. *Amblyomma americanum* is considered the main established tick species of central Oklahoma (Noden & Dubie 2017).

Oklahoma is one of the nation's leaders in occurrences of spotted fever group rickettsiosis and ehrlichiosis (Biggs et al. 2016). In 2017, 475 individuals were reported to the Oklahoma State Department of Health (OSDH) for acquiring a tick-borne disease and 431 of those reports were due to Rocky Mountain spotted fever and ehrlichiosis (OSDH 2018 https://www.ok.gov/health2/documents/Reportable%20Conditions,%202017.pdf). In the 1990s, Rocky Mountain spotted fever was reported among the population at an average of 55 cases per year in Oklahoma. However, in the following decade, an average of 167 cases were reported per year. The average increased in the years of 2010 to 2017 to 279 cases (OSDH 2017 https://www.ok.gov/health2/documents/History%20Table%201990-2017.pdf). An increase also was seen in disease occurrence of ehrlichiosis; although it was not a reportable disease by law in the 1990s. The average occurrence from 2000 to 2009 was 65 cases per year and from 2010 to 2017 the average rose to 96 cases per year (OSDH 2017 https://www.ok.gov/health2/documents/History%20Table%201990-2017.pdf).

The city of Edmond is located in Oklahoma County, in central Oklahoma. Currently, the only established tick species in Oklahoma County is *A. americanum* (Barrett et al. 2015), although *A. maculatum, D. variablilis,* and *I. scapularis* have been reported (Mitcham et al. 2017). Most studies do not find *R. sanguineus* ticks when sampling habitats in Oklahoma and throughout the U.S. However, this species has been reported as the second most frequent species obtained from dogs in central Oklahoma, although reports of this species parasitizing humans are rare (Raghavan et al. 2007). *R. sanguineus* also is known to transmit Rocky Mountain spotted fever. (OSDH 2017 https://www.ok.gov/health2/documents/History%20Table%201990-2017.pdf).

Each tick species has been reported to carry one or more different bacterial pathogens responsible for causing disease in humans (Table 2). These pathogens include *Francisella tularensis, Rickettsia rickettsii, R. parkeri, Borrelia burgdorferi, Anaplasma phagocytophilum, Ehrlichia chaffeensis,* and *E. ewingii* (https://www.cdc.gov/ticks/geographic_distribution.html). *Rickettsia amblyommii* is carried by *A. americanum*, however, it has not been confirmed to be pathogenic to humans and currently is considered a potential pathogen (Openshaw et al. 2010).

Most studies observing pathogen prevalence in Oklahoma pool ticks together instead of testing individual ticks. Recent studies in Oklahoma found approximately 70% of *A. americanum* pools had *Rickettsia spp.* and 10% had *E. chaffeensis* (Mitcham et al. 2018). Noden et al. (2016) had similar rates of *Rickettsia spp.,* however, *E. chaffeensis* was present at a rate of approximately 26%. Salazar (2015) observed pathogen prevalence among individual laboratory-reared ticks and wild ticks from state parks in Oklahoma. The wild ticks tested were all *A. americanum,* and 21 of 39 were positive for

Rickettsia spp. In addition, 1 of 39 ticks were positive for *E. chaffeensis.* Positive samples were sequenced and no *R. rickettsii* was found (Salazar 2015). In addition, Mixson et al. (2006) found an overall prevalence across nine states in the U.S. to be 4.7% for *E. chaffeensis* and 45.2% for *R. amblyommii* among *A. americanum* ticks. This study included 60 ticks from Payne County, Oklahoma, and found prevalence to be 3.3% for *E. chaffeensis* and 11.8% for *R. amblyommii* (Mixson et al. 2006). Although these studies provide foundational data for Oklahoma pathogen prevalence in *A. americanum* ticks, the small sample sizes may not accurately describe pathogen prevalence.

Residential vs. Non-Residential Studies

Recent studies suggest that ticks and tick-borne pathogens occur in urban environments during summer months, but current research lacks in providing year-round data on tick presence in urban areas of Oklahoma (Noden et al. 2016). In 2017, Rocky Mountain spotted fever and ehrlichiosis were reported in 12 months and tularemia in 10 months out of the year among the human population (OSDH 2018 https://www.ok.gov/health2/documents/Reportable%20Conditions,%202017.pdf). These monthly disease occurrences show that the risk for contracting a tick-borne disease is present in months beyond the summer season and with annual tick-borne disease occurrence increasing it is important to study ticks in all months of the year.

Current data suggests that ticks and tick-borne pathogens have the potential to flourish in urban landscapes. Two city parks in Little Rock, Arkansas, were sampled for ticks and pathogens belonging to the *Rickettsia* and *Ehrlichia* genera (Blanton et al. 2014). Collection occurred for three days in July of 2011 and 273 ticks were obtained

(Blanton et al. 2014). When testing for pathogens, 42 of 43 pools were positive for *Rickettisae* in *A. americanum* and 1 of 43 pools were positive for *Ehrlichia* (Blanton et al. 2014). Noden et al. (2016) collected 552 ticks from parks within an urban city in Oklahoma. Parks had differing degrees of surrounding development and pathogen prevalence among *A. americanum* was found at rates of 75% in the 48 pools for *Rickettsia spp.*, 31% in the 48 pools for *E. chaffeensis,* and 14.5% in the 48 pools for *E. ewingii* (Noden et al. 2016). These studies offer foundational data of pathogen presence; however, they do not provide an accurate prevalence rate of pathogens among individual ticks. Because ticks are pooled, the most I can assume is that at least one of the ticks in the pool was carrying the pathogen and as a result, an inaccurate representation of prevalence was obtained. In addition, minimal information exists on comparisons of tick presence and species diversity between residential and non-residential parks, although tick-borne disease occurrence in the human population has shown increased rates over the last 30 years in Oklahoma (OSDH 2017 https://www.ok.gov/health2/documents/History%20Table%201990-2017.pdf).

Over the past decade, there has been approximately an 8% increase in human population density for Oklahoma County (https://factfinder.census.gov/faces/nav/jsf/pages/community_facts.xhtml). Urbanization has occurred and, therefore, humans and their pets may be filling the role of hosts for tick vectors (Noden et al. 2016). In addition, deforestation can result in altered population dynamics of tick hosts and, therefore, have an impact on the tick populations present (LaDeau et al. 2015). Population structure studies on animals, such as white-tailed deer (*Odocoileus virginianus*), red fox (*Vulpes vulpes*), and Norway rat (*Rattus norvegicus*),

indicate successful adaptation of these animals to urban landscapes (Paddock and Yabsley 2007, Blanchong et al. 2013, Matuschka et al. 2016). Moreover, these species serve as hosts to ticks and reservoirs of pathogens. The tick species known to carry and transmit Rocky Mountain spotted fever (*R. rickettsii)*, the American dog tick and brown dog tick*,* are vectors found in urban areas that prefer to feed on domestic dogs in Oklahoma (Raghavan et al. 2007). The lone star tick, a vector for *Ehrlichia* and *R. amblyommii*, tends to feed on livestock and wildlife (Murphy et al. 1998). Because distribution of preferred tick hosts varies, it is essential to survey urban and rural environments to provide the potential public health risks of both settings. Noden et al. (2016) observed ticks and tick-borne pathogens in city parks with differing amounts of surrounding development. A majority of the ticks collected came from parks with more undeveloped land surrounding them (Noden et al. 2016). However, there was not a difference in pathogen prevalence among the tick populations (Noden et al. 2016).

Several hypotheses attempt to explain the increase in tick-borne disease occurrence, however, more research needs to be done. The dilution effect and host adaptation to urban landscapes offer potential explanations for the increase in disease occurrence. The dilution effect is the hypothesis that high species richness of tick hosts would result in fewer infected reservoir hosts and a decrease in pathogen prevalence (Schmidt & Ostfeld 2001, Bradley & Altizer 2007). Although there is a potential increase of the vectors due to increased host availability, there also would be a decrease in disease occurrence because fewer hosts being fed on by the ticks would have viable pathogens (Schmidt & Ostfeld 2001). Therefore, the pathogen would not be acquired by the tick.

This effect would only apply to pathogen species that rely on (1) horizontal transmission and (2) ticks feeding upon multiple host species (Schmidt & Ostfeld 2001).

Prominent Pathogens in Oklahoma

Rickettsia

Rickettsiae and *Ehrlichiae* are gram-negative, obligate intracellular organisms (Biggs et al. 2016). Both genera belong to the α subdivision of the Proteobacteria (Sonenshine & Roe 2014, p. 211). *Rickettsia* species are divided into four groups, in which three of the groups contain human pathogens consisting of the spotted fever group, typhus group, and transitional group (Walker & Ismail 2008). The causative agent responsible for Rocky Mountain spotted fever was discovered by Howard Ricketts in 1906 (Sonenshine & Roe 2014, p. 211). Currently, *R. rickettsii* is known to be transmitted by *D. variablilis* and *R. sanguineus,* however, it is not known whether *Amblyomma spp.* transmit or carry *R. rickettsii,* or other spotted fever group members in the United States (Biggs et al. 2016). In order for *Rickettsia spp.* to infect a host, they must first be injected into a host, attach to host cells, and invade the cells (Brooks et al. 2010, p. 349).

Reservoir hosts are responsible for maintaining pathogens in an environment and are key to horizontal transmission of pathogens by ticks. Lagomorphs and rodents, such as *Spermophilus lateralis* (golden-mantled ground squirrel)*, Microtus pennsylvanicus* (meadow vole)*, Eutamias amoenus* (chipmunk)*,* and *Lepus americanus* (snowshoe hare)*,* are the main reservoir hosts for *Rickettsia spp.* (Sonenshine & Roe 2014, p. 233). However, it also is suggested that because *Rickettsia* species are transmitted

transovarially, ticks can be considered reservoir hosts for the pathogens (Uchiyama 2012, Sonenshine & Roe 2014, p. 233).

Ticks obtain *Rickettsia spp.* by feeding on an infected host or by transovarial transmission. Once inside the midgut of the tick rickettsiae escape endosomes during a period of delayed lysosome formation and invade cells of the tick midgut. Rickettsiae replicate in the cytosol of the cells, migrate out of the cells, and invade other organs of the tick including the salivary glands and ovary. It is suggested that *Rickettsia* evade detection by the human immune system (Sonenshine & Roe 2014, p. 233).

Rickettsiae attach themselves via outer membrane protein A (OmpA) and OmpB to the endothelial cells of the vasculature system (Brooks et al. 2010, p. 351). OmpA interacts with integrins at the surface of the host cell and OmpB interacts with ku70 (Walker & Ismail 2008, Sahni 2013). Integrins are proteins on the surface of host cells and are responsible for the adhesion of cells to other cells or proteins (Abbas et al. 2012, p. 485). Ku70 is a 70 kDa protein that complexes with other proteins to form the DNA-protein kinase complex and acts as a receptor for OmpB (Chan et al. 2009). Following the attachment to the surface of vascular endothelial cells, *Rickettsiae* induce phagocytosis resulting in their engulfment by the cell (Liu et al. 2014). They use hemolysin and phospholipase to be released by the phagosome into the cytosol of the host cell (Liu et al. 2014). Although they can infect various cells, their affinity is for vascular endothelial cells (Sahni 2013). Upon entering the cell, *Rickettsiae* form a polar actin tail used for intracellular and intercellular movement, although the actin tail is present in varying degrees depending on the *Rickettsia* species (Sahni 2013). Members of

Rickettsiae multiply by binary fission and are able to infect adjacent cells by actin tail movement (Minniear & Buckingham 2009).

Upon the transmission of *R. rickettsii,* individuals become ill with a high fever accompanied by headache, nausea, and myalgia (Biggs et al. 2016). A rash often forms within the first few days of fever (2-4 days; Biggs et al. 2016). The immune system's response to *Rickettsiae* causes increased occurrence of lymphocytes surrounding the blood vessel resulting in edema (Brooks et al. 2010, p. 351). A rash is present in 95% of children and 80% of adults, however, the rash can be quite varied. The infection causes vascular permeability and increased fluids in interstitial space which can lead to shock, if not treated in a timely manner (Walker et al. 2017).

Rocky Mountain spotted fever had a case-fatality rate of 25% prior to the antibiotic era. However, the current clinical case-fatality rate is 5-10% of individuals, although the rate increases with delayed treatment (Biggs et al. 2016). Delays in treatment occur most commonly due to misdiagnoses (Minniear & Buckingham 2009).

Ehrlichiae

Human monocytotrophic ehrlichiosis (HME), caused by *E. chaffeensis* and *E. ewingii,* was first diagnosed in 1986 in a 50-year-old male with several tick bites and in critically ill condition (Paddock & Yabsley 2007). At the time, this genus was only thought to be of veterinary importance (Paddock & Yabsley 2007). These two pathogens are transmitted by *A. americanum,* which is a dominant tick species in areas of increased Ehrlichiosis occurrence (Jerrard 1999). HME has been described as the most life threatening vector-borne disease in the U.S., with approximately half of diagnosed

patients becoming hospitalized and death occurring as quickly as the second week of illness (Heitman et al. 2016). HME is a multisystem disease that, when diagnosis is delayed, results in severe complications (Heitman et al. 2016).

Odocoileus virginianus is the reservoir host for *E. chaffeensis* and a primary host for *A. americanum* ticks (Eisen et al. 2017). In addition, there have been surveys on the prevalence of *Ehrlichia* among dogs and coyotes in Oklahoma to determine additional reservoir hosts (Murphy et al. 1998, Kocan et al. 2000). Kocan et al. (2000) reported 15 of 21 coyotes in Oklahoma tested positive for *Ehrlichia* and, because coyotes tend to have a greater home range than deer, they could spread this pathogen to domestic animals, such as dogs, which can be secondary reservoirs (Kocan et al. 2000, Rikihisa 2010).

Ticks obtain *Ehrlichia* by feeding on an infected host, however, transovarial transmission is ineffective for this organism (Bakken & Dumler 2000). *Ehrlichia* species have two stages, a dense-cored cell and a reticulate cell (Rikihisa 2010). In ticks, *Ehrlichia* cells, in the dense-core form, invade epithelial cells of the midgut where they transform to the reticulate state and form large colonies (Sonenshine & Roe 2014, p. 253-254). They divide via binary fission and transform into the dense form upon exiting the cell (Sonenshine & Roe 2014, p. 254). This form infects the salivary glands, among other tissues, of the tick and they are then injected with tick saliva into a host upon feeding (Jerrard 1999, Sonenshine & Roe 2014, p. 254).

Recent studies have shown that for *Ehrlichia* to enter a eukaryotic cell, it uses an adhesin on the cell surface to bind a receptor of the host cell to initiate endocytosis of the organism by the host cell (Kumar et al. 2013). It also is thought that once within the host

cell, *Ehrlichia* bind to endosomes with transferrin receptors in the cell (Rikihisa 2010). The reticulate state occurs intracellularly and divides by binary fission (Sonenshine & Roe 2014, p. 254). The dense-cored state survives extracellularly and is the infective form that enters monocytes by endocytosis of the cell (Sonenshine & Roe 2014, p. 252). *Ehrlichiae* infect monocytes where they multiply within phagocytic vacuoles and form morulae (clusters) in the reticulate state (Brooks et al. 2010, p. 353). Upon entering the cell, the organism alters the intracellular signaling and stops the phagosomal maturation process, allowing for the organism to evade degradation (Holtom 2008, p. 1168). In addition, host cell respiratory burst is down regulated and cellular apoptosis is delayed (Holtom 2008, p. 1167). As division of the reticulate cells continues, the host cell either ruptures or organisms undergo exocytosis (Rikihisa 2010). The released ehrlichial cells then attach to other monocytes and continue the cycle (Rikihisa 2010).

The incubation period for HME is five to 21 days and symptoms include fever, swollen lymph nodes, chills, headache, myalgia, nausea, and weight loss; rarely is a rash associated with this disease (Olano et al. 2003). Diagnosis for this organism is confirmed by using the Wrights stain to visualize the morulae in white blood cells (Figure 1; Bakken & Dumler 2000, Brooks et al 2010, p. 354). Although the gold standard is cultivation of the organism, this process can take over a month and treatment should not be delayed (Jerrard 1999).

Current treatment for ehrlichiosis and Rocky Mountain spotted fever (RMSF) consists of tetracyclines, specifically doxycycline (Botelho-Nevers et al. 2012). Tetracyclines were discovered in the 1940's and are broad-spectrum antibiotics (Chopra & Roberts 2001). They are bacteriostatic; they rid the host of the infection by inhibiting

multiplication of the bacteria (Brooks et al. 2010, p. 60, Botelho-Nevers et al. 2012). Chloramphenicol was initially considered to be as effective as tetracyclines; however, studies revealed that the death rate due to RMSF was higher in patients treated with chloramphenicol (Holman et al. 2001). In addition, tetracyclines, specifically doxycycline, have a low minimum inhibitory concentration (MIC) against *Rickettsiae* and *Ehrlichiae* (Wisseman & Waddell 1982, Brouqui & Raoult 1992). As stated previously, both *Rickettsia spp.* and *E. chaffeensis* are gram negative organisms, therefore, tetracyclines must cross the membrane of the infective cells (Chopra & Roberts 2001). Gram negative bacteria have porin channels, which tetracyclines use to enter the cells; specifically channels OmpF and OmpC (Chopra & Roberts 2001). Upon entering the cell, tetracyclines bind to the 30s ribosomal subunit and block the binding site of aminoacyl-tRNA, therefore, tetracyclines prevent protein synthesis in bacteria (Brooks et al. 2010, p. 394). Side effects include significant risks to fetal bone formation in pregnant women and dental staining of children under the age of 6 when treated with tetracyclines, therefore, a balance of infection severity and dosage amount must be considered (Minniear & Buckingham 2009, Brooks et al. 2010, p. 394).

Purpose

The purpose of this project is to provide extent and distribution of ticks and tick-borne pathogens in two public parks. I also wanted to compare parks with increased surrounding development to parks with less surrounding development to determine if there is a difference in pathogen prevalence. Ticks were collected from June of 2016 to June of 2017 in woodland and grassland habitats of two public parks in Edmond,

Oklahoma. Of the two parks selected for this project, one was in an area of Edmond zoned as residential and the other was in a non-residential area. Collected ticks were tested by multiplex quantitative real-time polymerase chain reaction (qPCR) to determine presence or absence of *Rickettsia spp.* and *E. chaffeensis* among individual tick samples.

Chapter 2 of this document was formatted for submission to the Journal of Vector Ecology and currently is under review. Chapter 3 of this document was formatted for submission to the Journal of Vector-Borne and Zoonotic Diseases and will be submitted in the spring of 2019.

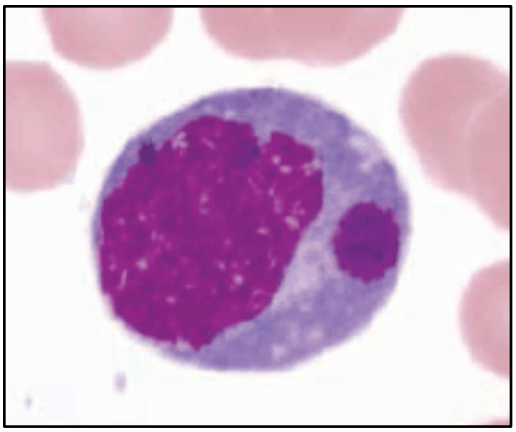

Figure 1. Wright Stain of a blood smear showing an *Ehrlichia chaffeensis* morula within a monocyte (Adapted from Biggs et al. 2016).

Chapter 1 Tables

Table 1: Ixodid tick species and their distribution across the United States as described by Nieto et al. (2018).

Species	Region of the United States
Amblyomma americanum	Midwest, Northeast, South
Amblyomma cajennense	Midwest, South
Amblyomma maculatum	South
Dermacentor andersoni	West
Dermacentor occidentalis	West
Dermacentor variabilis	West (coast), Midwest, Northeast, South
Ixodes pacificus	West
Ixodes scapularis	Midwest, Northeast, South
Rhipicephalus sanguineus	West, South

Table 2: Human tick-borne pathogens, diseases they cause, and tick species that transmit each pathogen in Oklahoma.

Pathogen	Disease	Tick Species (Common name)
Anaplasma phagocytophilum	Anaplasmosis	*Ixodes scapularis* (Deer)
Borrelia burgdorferi	Lyme Disease	*Ixodes scapularis* (Deer)
Ehrlichia chaffeensis	Ehrlichiosis	*Amblyomma americanum* (Lone star)
Ehrlichia ewingii	Ehrlichiosis	*Amblyomma americanum* (Lone star)
Francisella tularensis	Tularemia	*Amblyomma americanum* (Lone star), *Dermacentor variabilis* (American dog)
Rickettsia amblyommii	Spotted Fever Group Rickettsiosis	*Amblyomma americanum* (Lone star), *Dermacentor variabilis* (American dog)
Rickettisa parkeri	Spotted Fever Group Rickettsiosis	*Amblyomma maculatum* (Gulf Coast)
Rickettsia rickettsii	Spotted Fever Group Rickettsiosis	*Dermacentor variabilis* (American dog), *Rhipicephalus sanguineus* (Brown dog)

CHAPTER 2

Seasonal Sampling of Ticks in Residential and Non-Residential Parks in Edmond, Oklahoma, Identifies Novel Establishment of Species

INTRODUCTION

Ticks and tick-borne pathogens have a worldwide distribution and impact the medical, veterinary, and cattle industries (Brites-Neto et al. 2015). In recent years, Oklahoma has seen an increase in tick-borne disease occurrence in the human population, although minimal research exists on the distribution of ticks throughout residential parks or changes in tick abundance on an annual basis (Biggs et al. 2016).

Preliminary tick collections resulted in only hard ticks, therefore, members of Ixodidae are the focus of this study. Ixodidae spend more than 90% of their lives off of a host, which results in their life cycle being greatly affected by climate (Needham and Teel 1991). Temperature and relative humidity directly impact survival and developmental rates (Ogden et al. 2005). In Oklahoma, *Amblyomma americanum* (lone star) adults and nymphs had a survival rate of over 90% when temperatures were between 12^{o}C and 30^{o}C, and relative humidity was between 65% and 85% (Koch 1984). When temperatures were below 10^{o}C, adult survival remained at 90%, but nymph survival decreased to approximately 60% (Koch 1984). Adult ticks are better adapted to survive changing environmental conditions than younger life stages; therefore, developmental time can affect survival rates and changes to tick population density (Koch 1984).

A large portion of tick research focuses on the months between April and October, resulting in a lack of information on tick presence and abundance in the winter.

Oklahoma state parks have been surveyed for ticks and tick-borne diseases in the months of April through August, and three tick species were obtained by flagging vegetation (Mitcham et al. 2018). Recent studies also provided evidence of four tick species in urban habitats during summer and fall months in Oklahoma (Noden et al. 2016). County-wide surveys confirmed the presence of established *A. americanum* populations in 68 of 77 Oklahoma counties (Barrett et al. 2015). Mitcham et al. (2017) defines an established tick species as at least six ticks or two different life-stages collected in a single trapping period. In Oklahoma County, *Dermacentor variabilis* (American dog), *Ixodes scapularis* (deer), and *A. maculatum* (Gulf Coast) have been identified as reported populations and *A. americanum* as an established tick population (Mitcham et al. 2017).

Because tick presence is dependent upon host availability, habitat, and abiotic conditions, environmental changes have the potential to influence tick and tick-borne pathogen presence (Ogden and Lindsay 2016). Only in recent years have studies addressed tick populations and tick-borne pathogens in urban areas (Noden et al. 2017). The impacts of urbanization can cause fluctuations to host abundance and availability, host distribution, and species prevalence, ultimately impacting tick presence and survival (Ogden et al. 2005, LaDeau et al. 2015).

The goal of this year-long study was to quantify tick presence, abundance, and species diversity in residential and non-residential parks within an urban city in Oklahoma County, Oklahoma. This study provides baseline quantification of tick abundance and distribution for a full year, and insight into the potential risk of encountering different tick species in all seasons in residential and non-residential parks.

MATERIALS AND METHODS

Study Area

Tick collection occurred at Mitch Park and Edmond Park in Edmond, Oklahoma (Figure 1). Edmond is an urban city with a population of approximately 90,000 that covers 219 km^2 (United States Census Bureau 2016). Average rainfall for this area during 2016 and 2017 was 76 cm to 100 cm and average temperature ranges were 0^oC to 32^oC. Climate data was obtained from Mesonet (Brock et al. 1995, McPherson et al. 2007). For this study, residential and non-residential parks were defined by the Edmond City Zoning and Planning Committee (https://gis.edmondok.com/plzoning/). The residential areas in Edmond are defined as areas with single-family dwellings, and related recreational, educational, and religious facilities.

Mitch Park (Figure 2), zoned as residential, is west of I-35 and is a 113-hectare plot with recreational amenities that allows access to the public year-round. It consists of trails leading around the park and to near-by neighborhoods, multiple playgrounds, baseball fields, and a disc golf course. Edmond Park (Figure 2), zoned as agricultural, is east of I-35 and is 53 hectares with public access through the spring and summer months. Edmond Park contains hiking trails, primitive camping spots, and access to Arcadia Lake.

Collection Methods

Woodland and grassland habitats were sampled at each park using dry-ice and drag collection methods continuously for 13 months beginning in June of 2016. Dry ice traps and tick drag methods were used for collection. Dry ice traps were set along transects and constructed with 30.5 x 30.5 cm cardboard squares with masking tape

around the perimeter (Barrett al. 2015). Three grassland and three woodland transects at each park were numbered and a random number generator was used to select two woodland and two grassland transects on each collection day, with five traps placed along each transect and at least 4.5 m apart. Transects ran parallel to park trails, roads, or areas with human amenities such as parking lots, playgrounds, and buildings. GPS coordinates were obtained for each transect (Appendix I). Dry ice was placed at the center of the traps, which were left in place for 90 minutes. Sampling occurred for one continuous year beginning in June 2016 and each park was sampled once per month. Tick drags were used in grassland areas during the 90 minutes traps were set, dragging occurred at a minimum of 30 m away from tick traps, and the amount of time spent using the drags was recorded (Dantas-Torres et al. 2013). Following collection, adult tick species were identified by their mouth parts and coloration (Keirans and Litwak 1989). A stereo microscope (Olympus SZ51) was used to identify nymphs by their mouth parts, shape, and coloration (Keirans and Durden 1998). In addition, sex and life stage were recorded and ticks were placed in 96 well plates with 70% ethanol, followed by -20^{o}C storage.

Data Analysis

To better understand tick presence at each park, I applied a binomial generalized linear mixed-model using the R (R Core Team 2018) package 'lme4' (Bates et al. 2015) with the 'glmer()' function to predict presence of adult or nymph ticks at a trap. I treated park (Mitch Park or Edmond Park) and location within park (woodland or grassland habitat) as fixed effects and accounted for the random effect of individual traps being grouped along trapping transects. Captures of adult ticks (combined male and female

totals) and unsexed nymph ticks were included for each capture opportunity, resulting in 1440 opportunities to document the presence of an adult or nymph stage tick.

RESULTS

Tick Collection Totals and Seasonal Observations

A total of 2,450 ticks were collected over the year, with approximately twice as many ticks collected from the residential park than the non-residential park (Appendix II). At least 90% of the ticks collected at each park were *A. americanum* (Table 1) and, at both parks, four tick species were collected over the year. *Americanum americanum* ticks were collected during all months of the year, with peak presence occurring in the summer months for both males and females (Figure 3). At both residential and non-residential parks, more female *A. americanum* ticks were collected than males. *Dermacentor variabilis* and *A. maculatum* ticks were collected only in the spring, summer, and fall (Figure 4). The first *I. scapularis* was collected in October and the last in March (Figure 4).

Establishment of Species

Amblyomma maculatum were more common in the grassland habitats than the other species collected. Traps placed in the woodland habitats had fewer occurrences of zero ticks collected than the grassland habitats. *Amblyomma americanum* ticks were the most commonly caught species in the woodland habitat, followed by *D. variabilis*, *A. maculatum* and *I. scapularis*. In July and March of 2016, and May of 2017, greater than six *D. variablilis* were collected at either or both parks. *Amblyomma maculatum* occurred

in abundances greater than six in the months of June of 2016 and May of 2017. Of the *I. scapularis*, six or more were collected in the late fall and winter months; November and December of 2016, and January of 2017.

Amblyomma americanum Presence

At both parks, the majority of traps sampled had between one and 30 *A. americanum* tick captures, however, the residential park (Mitch Park) more frequently had counts in excess of 30, specifically nymphs (Figure 5a and 5b). Tick presence was lower at the non-residential park (Edmond Park), in both woodland and grassland habitats, than Mitch Park (Figure 6, Table 2). Tick collection was lower in grassland habitats, but when ticks were present on a trap, the number of ticks was between one and 10. *Amblyomma americanum* presence was significantly lower in grassland areas relative to woodland areas (GLMM; Table 2). The calculated entry 0.3980 in Table 3 indicates 39.80% of traps in woodland parks of Mitch Park (residential park) were expected to yield one or more ticks.

DISCUSSION

Oklahoma is one of the nation's annual leaders in human tick-borne disease occurrence (Eisen et al. 2008, Drexler et al. 2016, Heitman et al. 2016). Therefore, determining presence of ticks in residential and non-residential parks in a highly populated county of Oklahoma is important in understanding potential tick exposure risk to humans and their pets. By sampling in different landscapes and habitats, I provide

important baseline information for the presence, abundance, and spatial distribution of tick species during all seasons of the year.

Each species was collected in greater abundance at the residential location (Table 1). This could be caused by host prevalence and abundance, habitat, weather conditions, and trapping methods (Koch 1984, Perkins et al. 2006, Kilpatrick et al. 2014). Pesticides are not used routinely at either park (C. Dishman, Director, Edmond City Parks and Recreation, pers. comm, 2 August 2018). However, the residential park is surrounded by a school and neighborhoods, which may have more pesticide use, potentially resulting in a concentrated area of tick populations in the park. In addition, primary tick hosts, such as the white-tailed deer, have been found to use urban landscapes and their activity may impact tick prevalence (Grund et al. 2002, Kilpatrick et al. 2014). To better understand host presence in this landscape, studies involving host abundance and presence are urgent. Approximately 91% of the total ticks were obtained from the woodland habitats. This could be due to the cooler temperatures and higher humidity resulting in less desiccation of the ticks than in the more open grassland habitat. Koch (1984) found that *A. americanum* survival rates were lowest in a meadow habitat compared to two forested habitats.

Amblyomma americanum ticks were present every month at both parks (Figure 3). Its primary host is the white-tailed deer and it transmits the human pathogens *Rickettsia spp., Ehrlichia spp.,* and *Francisella tularensis* (Barrett et al. 2015). *Amblyomma americanum* nymphs were collected in all months of the year, with the greatest occurrences being in May and June (Figure 3). Bouzek et al. (2013) found host seeking activity among *A. americanum* nymphs to begin in March and continue through mid-

August, with peak activity occurring in June through August. Their data also suggested that temperatures of the previous year had a direct impact on the number of nymphs and adults present at the sampling time, and that temperature impacts developmental rates (Bouzek et al. 2013).

The percentage of each species collected in this study is consistent with results obtained by collecting ticks from horses in central Oklahoma and from state parks across Oklahoma (though sampling strategies were different). From horses in central Oklahoma, 1,721 ticks were obtained and *A. americanum* constituted over 92% of the ticks collected; *A. maculatum* composed 2%, 5% were *D. variablilis,* and less than 1% were *I. scapularis* and *D. albiticus* (winter) (Duell et al. 2013). However, this data was obtained only for May through July 2010. Mitcham et al. (2018) collected 1,035 ticks by flagging vegetation from April through August 2014. *Amblyomma americanum* comprised approximately 94% of total captures, *D. variabilis* 3.5%, and *I. scapularis* 2% (Mitcham et al. 2018). Previously, it was thought that tick response to dry ice traps varied based on trapping conditions, relative humidity, wind speed, and temperature (Koch 1987). However, in my study I obtained similar results to Mitcham et al. (2018) using dry ice traps; *A. americanum* at 91.5%, *A. maculatum* at 2%, *D. variablilis* at 2%, and *I. scapularis* at 0.5%; the remaining 4% were collected by dragging (Table 1).

This study also demonstrated the presence and absence of ticks in differing months of the year. In addition to *A. americanum,* which I have established was present in every month, *A. maculatum* was present in six months, *D. variabilis* in eight months, and *I. scapularis* in six months. Distribution maps of active and passive surveillance of tick species among Oklahoma counties has occurred in recent years and species have

been identified as reported or established, although these studies did not utilize year-round surveillance (Barrett et al. 2015, Mitcham et al. 2017). Sampling for this study occurred in Oklahoma County and confirmed established populations of not only *A. americanum,* but also *A. maculatum, D. variablilis,* and *I. scapularis.* If tick collection was restricted to April through August, *I. scapularis* would not have been captured and confirmed as an established population in Oklahoma County. This species is responsible for the transmission of *Borrelia burgdorferi*, the causative agent of Lyme disease which causes approximately 30,000-40,000 people to fall ill on an annual basis in the U.S. (Adams et al. 2017). Although Oklahoma has few cases of confirmed Lyme disease per year, without accurate representation of this tick population, the prevalence and potential risk of encountering this disease will remain unknown in Oklahoma (Adams et al. 2017). In addition, seasonality of *A. americanum* and *D. variabilis* is underrepresented in the literature and lacks in providing the potential for encountering ticks in the winter months.

In conclusion, ticks were found to be abundant in residential and non-residential parks, and active in all months of the year in an urban area in Oklahoma. Although this study consisted of one year of sampling, it provides foundational data for future studies. More annual data could provide a better understanding of the presence of tick populations in residential and non-residential parks. This project can be used to better inform the Edmond population of where and when ticks are commonly found in these public spaces. Future research should aim to better understand the relationship between tick populations and residential habitats, in addition to tick species seasonality in Oklahoma.

Acknowledgements

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Chapter 2 Figures

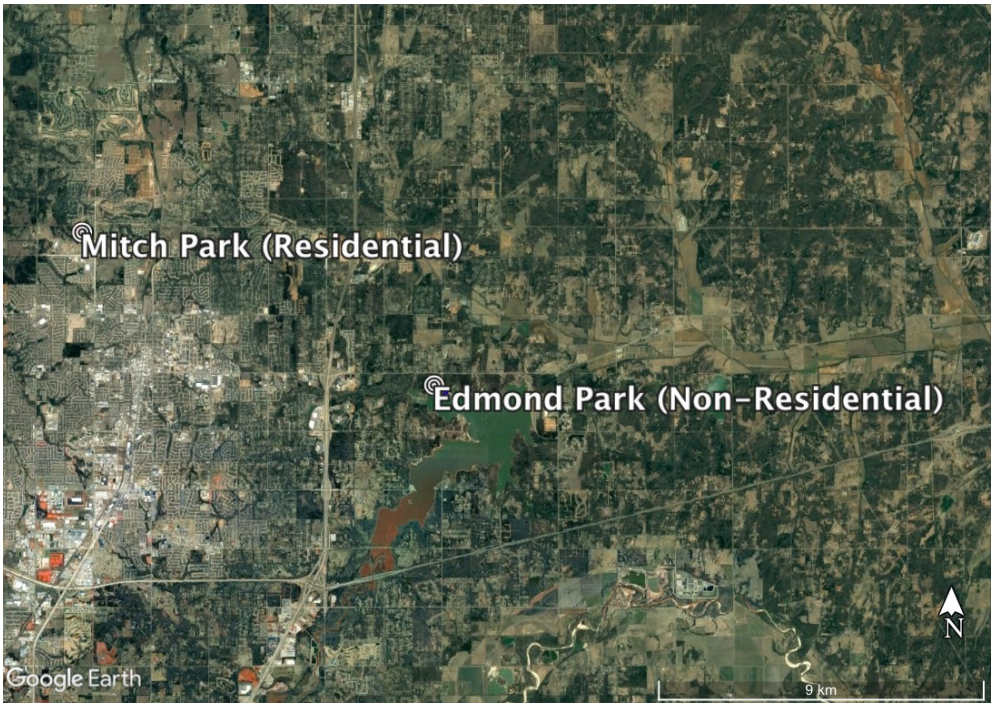

Figure 1. Residential and Non-Residential Parks in Edmond, Oklahoma (Google Maps, 2018), sampled during this study.

Figure 2. Residential park (Mitch Park) and non-residential park (Edmond Park) (Google Maps, 2018), each with woodland (blue) and grassland (red) transects marked.

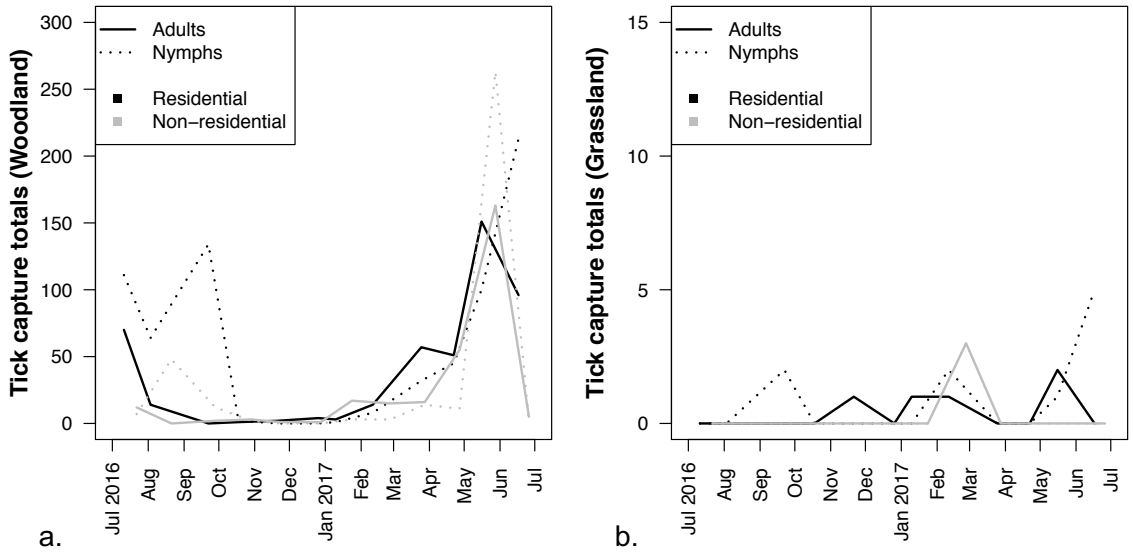

Figure 3. *Amblyomma americanum* tick capture totals in woodland (3a) and grassland (3b) parks. On figure 3b, solid and dotted gray lines coincide near March 2017, indicating an equal number of adult and nymph ticks.

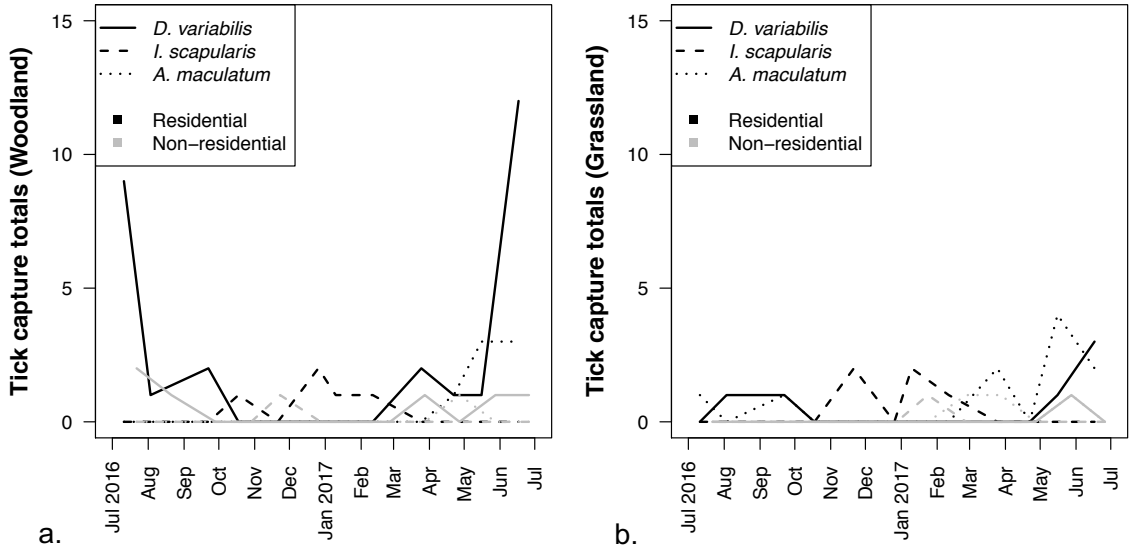

Figure 4. Capture totals for non-*A. americanum* tick species in woodland (4a) and grassland (4b) parks at Mitch Park and Edmond Park in Edmond, Oklahoma.

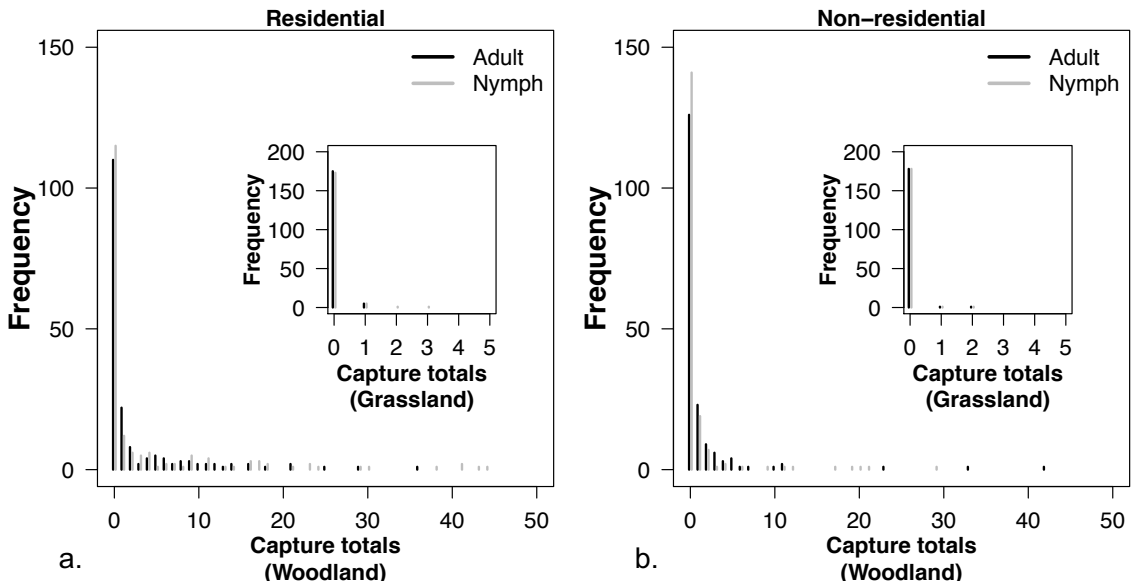

Figure 5. Frequency of total captures for adult (black bars) and nymph (gray bars) *A. americanum* ticks in woodland and grassland parks at Mitch Park (5a) and Edmond Park (5b). Grassland total captures are inset due to fewer captures of adult and nymph *A. americanum* ticks.

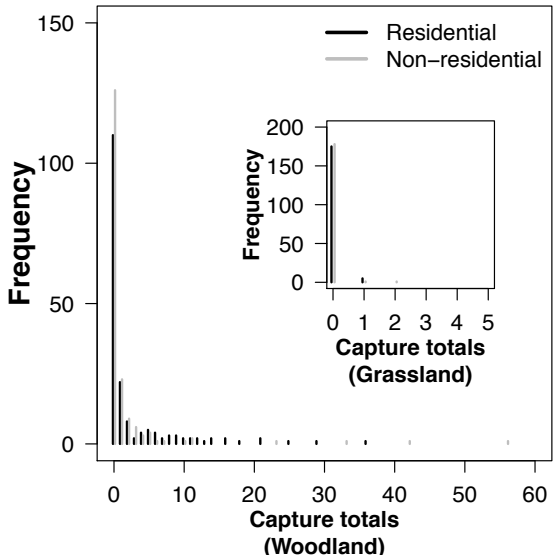

Figure 6. Capture data for adult *A. americanum* ticks only at Mitch Park (black) and Edmond Park (gray). Grassland total captures are inset due to fewer captures of adult and nymph *A. americanum* ticks.

Chapter 2 Tables

Table 1. Tick species collected at residential and non-residential parks based on two trapping methods in Edmond, Oklahoma.

Park	*Dermacentor variabilis*	*Ixodes scapularis*	*Amblyomma maculatum*	*Amblyomma americanum*	Total
Non-Residential	24 (3%)	15 (1.5%)	4 (0.5%)	807 (95%)	850
Dry Ice Trap	10	2	4	802	818
Drag	14	13	0	5	32
Residential	46 (3%)	49 (3%)	57 (4%)	1448 (90%)	1600
Dry Ice Trap	38	10	44	1444	1536
Drag	8	39	13	4	64
TOTALS	**70**	**64**	**61**	**2255**	**2450**

Table 2. Estimates for GLMM predicting presence of *A. americanum* ticks at traps by park (Mitch Park or Edmond Park) and location (woodland or grassland habitat) with trap transect as a random effect. Log-likelihood of the model estimates is −497.7, AIC = 1007.4, BIC = 1039.0, and variance explained by transect is 0.615. Asterisks represent significance.

	Estimate	Std. Error	*z*-value	Pr(> |*z*|)	
Intercept	-0.4139	0.4725	-0.876	0.381	
Nymph (stage)	-0.2260	0.1581	-1.429	0.153	
Edmond Park (park)	-0.7463	0.6666	-1.119	0.263	
Grass (location)	-3.2386	0.7529	-4.301	1.7×10^{-5}	***
park-by-location interaction	-0.2315	1.1197	-0.207	0.836	

Table 3. Raw and calculated probabilities of *A. americanum* presence by park and location. Calculated probabilities use model estimates from Table 2.

	Mitch Park		Edmond Park	
	Woodland	Grassland	Woodland	Grassland
Adult presence	0.3889	0.0278	0.3000	0.0111
Calculated	0.3980	0.0252	0.2386	0.0097
Nymph presence	0.3611	0.2167	0.0389	0.0111
Calculated	0.3453	0.2000	0.0203	0.0077

CHAPTER 3

Tick-Borne Pathogens of Two Parks in an Urban City, Edmond, Oklahoma

Introduction

Rickettsial and ehrlichial disease occurrence has been increasing among humans in the United States over the past few decades (Biggs et al. 2016). However, few studies have examined pathogens in urban environments or city parks, and almost no studies have surveyed ticks in the winter months (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) is an established tick species in 68 out of 77 counties in Oklahoma (Barrett al. 2015). *Amblyomma americanum* is a pest across most of the Southeastern U.S. and transmits *Rickettsia spp., Francisella tularensis, Ehrlichia chaffeensis, E. 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).

The majority of pathogen prevalence studies group ticks together and test them as a pool. 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 for a full year in two Edmond, Oklahoma, parks (Chapter 2) were tested for *Rickettsia spp.* and *E. chaffeensis* by multiplex quantitative polymerase chain reactions (qPCR). The two parks were in areas with different city zoning, residential and agricultural. Ticks were collected from June of 2016 through June of 2017 and stored in 70% ethanol at -20^{0}C until qPCR testing.

Methods

Adult and nymph *A. americanum* ticks were dissected individually and tissues, including the midgut, were removed with a #11 scalpel (Varela-Stokes 2007). The tissues were placed into individual centrifuge tubes with lysis buffer and incubated at room temperature for 12 to 18 hours (Halos et al. 2004). A GE Illustra extraction kit (GE Healthcare Life Sciences, Pittsburgh, PA) was used to extract DNA from individual ticks. An alternative lysis buffer (NaCl 0.1M, Tris-HCL 0.21M, pH8 EDTA 0.05M, SDS 0.5%) was used in place of the buffer provided by GE; the remaining extraction procedure followed the protocol provided by the manufacturer (Halos et al. 2004). Extracted DNA was stored at -20^{o}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 *E. chaffeensis* and *Rickettsia amblyommii*, using previously described methods and primers (Gaines et al. 2014). A 50 µl total volume reaction was used and included Bio-Rad iQ Multiplex Powermix with 10 µl of DNA. Sequencing was performed on five random positive samples and confirmed *R. amblyommii* and *E. chaffeensis.* Primers used for sequencing

were from previously published data (Blair et al. 2004, Kocan et al. 2000). Chi-square tests were performed in R (R Core Team 2018) and used to determine statistically significant differences in pathogen prevalence within and between the two parks (R Core Team 2018).

Results

Of the 434 *A. americanum* ticks tested, *R. amblyommii* was the most prevalent pathogen in adults and nymphs at both sites, with a prevalence of approximately 50% (Table 1). *Ehrlichia chaffeensis* was most prevalent in the adult *A. americanum* ticks at the non-residential site. Statistical analyses showed no significant difference in pathogen prevalence between the two parks (data not shown). However, there was a significant difference in prevalence of pathogens within each park ($p < 0.05$). The prevalence of *R. amblyommii* was significantly higher than the other pathogens at each park. Of the *A. americanum* ticks tested, 14 were positive for *R. amblyommii* and *E. chaffeensis.*

Discussion

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 non-residential park provides access to Arcadia Lake, in addition to camping spots, hiking trails, and mountain biking trails. At both sites, the two pathogen species were found in both adult and nymph *A. americanum* ticks. *Rickettsia spp.* can be transmitted transovarially and transstadially (Biggs et al. 2016), therefore, I suspect that *A. americanum* may be acting as a reservoir host and maintaining the prevalence of this

pathogen. *Rickettsia* species were found to have a higher prevalence rate in nymph *A. americanum* ticks tested than adults. *Ehrlichia chaffeensis* is conserved by ticks transstadially, but not transovarially (Blanton et al. 2014). 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). *Ehrlichia chaffeensis* had its highest prevalence rate among *A. americanum* adults at the non-residential site. White-tailed deer populations may be more abundant in and surrounding the non-residential site. Grund et al. (2002) found that within a season, a white-tailed deer's home range consisted mostly of a park woodland area (similar to the non-residential site in this study). In contrast, in a residential woodland (similar to my residential park), a white-tailed deer's home range within that area was smaller (Grund et al. 2002).

Rocky Mountain spotted fever and ehrlichiosis are potential life-threatening diseases among the human population (Biggs et al. 2016). Therefore, it is important to inform the general public of areas where these pathogens 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 *Dermacentor variabilis* (American dog tick)*, Amblyomma maculatum* (Gulf Coast tick)*,* and *Ixodes scapularis* (deer tick) would provide more information on the potential for encountering additional tick-borne pathogens.

Acknowledgments

I would like to thank the University of Central Office of High Impact Practices and OK-INBRE for their grant awards. I would also like to thank Dr. Susan Little and Mr. Jeff Gruntmeir at Oklahoma State University for providing *E. chaffeensis* control DNA and Dr. Jere McBride from the University of Texas Medical Branch at Galveston for providing *R. amblyommii* control DNA.

Chapter 3 Table

Table 1: Pathogen prevalence among *A. americanum* ticks at a residential and non-residential park.

Park	Stage (# of Ticks tested)	*R. amblyommii* (# of Positive ticks)	*E. chaffeensis* (# of Positive ticks)
Residential	Adults (144) Nymphs (145)	33% (48) 37% (53)	14% (21) 13% (19)
Non-Residential	Adults (93) Nymphs (52)	29% (27) 35% (18)	22% (20) 11.5% (6)
Total	434	50% (146)	15% (66)

CHAPTER 4

SUMMARY AND CONCLUSIONS

In an effort to better understand tick and tick-borne pathogen prevalence in urban environments, I sampled ticks from a residential and non-residential park in Edmond, Oklahoma. Ticks were collected over one continuous year to provide foundational data on tick presence year-round. I also sampled in woodland and grassland habitats to better understand tick distribution and at-risk areas where people and their pets may encounter ticks in a public park. I hypothesized that there would be a significant difference in pathogen prevalence between the residential and non-residential parks. This study used methods of fieldwork and genetic analyses to determine tick presence and pathogen prevalence.

To determine tick presence, ticks were collected from Mitch Park (residential) and Edmond Park (non-residential) in Edmond, Oklahoma. Dry-ice traps were used to collect ticks in woodland and grassland areas at both sites. In addition, tick drags were used in grassland areas to sample questing ticks. Each park was sampled once per month from June of 2016 through June of 2017. Mitch Park is located on the west side of I-35 and is surrounded by neighborhoods, a school, and has amenities such as, walking trails, a disc golf course, and a playground. In contrast, Edmond Park is on the east side of I-35 and provides access to Arcadia Lake, camping spots, and hiking and mountain bike trails. Mitch Park is accessible to the public on a year-round basis, however, Edmond Park is closed from November to February. Both parks do not have routine pesticide use and Edmond Park had an estimated attendance of approximately 40,000 visitors in 2017 (C.

Dishman, pers. comm. 2 August 2018). Data on park attendance at Mitch Park is not currently available.

Over the year, 2,450 ticks were collected and consisted of four different species responsible for causing disease among humans. Prior to this study, the only established tick species in Oklahoma County was *Amblyomma americanum* (lone star). However, I have determined the remaining species to now be established populations in Oklahoma County. These species consist of *Amblyomma maculatum* (Gulf Coast tick)*, Dermacentor variablilis* (American dog tick)*,* and *Ixodes scapularis* (deer tick)*.* Had trapping occurred only during months of peak tick activity (May-August), I would not have collected *I. scapularis* and would have missed the seasonal shift and appearance or reappearance of species.

Ticks were dissected individually and analyzed for pathogen prevalence. Because Rocky Mountain spotted fever and ehrlichiosis are the most commonly reported tick-borne diseases in Oklahoma, my study focused specifically on prevalence of the main pathogens responsible for causing these diseases in humans. I used multiplex quantitative real-time polymerase chain reaction (qPCR) to determine presence or absence of the pathogens in 434 *A. americanum* ticks collected from both sites. I found overall prevalence rates to be approximately 6% for *R. rickettsii,* 29% for *R. amblyommii,* and 15% for *E. chaffeensis.* Although there was not a significant difference of pathogen prevalence between the two parks, there was a significance difference between pathogen prevalence within each park ($p < 0.050$). Surprisingly, 21 ticks had more than one pathogen present.

Few studies have tested individual ticks to determine pathogen prevalence or surveyed ticks on a year-round basis. Therefore, this study provides new insights regarding the presence of ticks in public parks and pathogen prevalence among *A. americanum* ticks in Oklahoma. This data will assist the parks and recreation department of the city of Edmond in better understanding tick populations in public parks and the potential risks to the general public attending these parks.

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APPENDIX I

Table 1. GPS coordinates for trapping transects at Mitch Park in Edmond, Oklahoma. (W=Woodland, G=Grassland)

	MITCHW1	MITCHW2	MITCHW3	MITCHG1	MITCHG2	MITCHG3
1ST POINT	N35, 41.335' W97, 29.871'	N35, 41.117' W97, 29.891'	N35, 41.176' W97, 30.120'	N35, 41.327' W97, 30.108'	N35, 41.322' W97, 30.030'	N35, 41.176' W97, 30.120'
2ND POINT	N35, 41.327' W97, 29.847'	N35, 41.189' W97, 29.929'	N35, 41.152' W97, 30.122'	N35, 41.337' W97, 30.063'	N35, 41.316' W97, 30.077'	N35, 41.250' W97, 30.163'

Table 2. GPS coordinates for trapping transects at Edmond Park in Edmond, Oklahoma. (W=Woodland, G=Grassland)

	ARCW1	ARCW2	ARCW3	ARCG1	ARCG2	ARCG3
1ST POINT	N35, 38.928' W97, 23.230'	N35, 38.875' W97, 23.268'	N35, 38.867' W97, 23.311'	N35, 38.921' W97, 23.264'	N35, 38.894' W97, 23.281'	N35, 38.867' W97, 23.311'
2ND POINT	N35, 38.952' W97, 23.236'	N35, 38.872' W97, 23.290'	N35, 38.863' W97, 23.351'	N35, 38.905' W97, 23.291'	N35, 38.889' W97, 23.316'	N35, 39.042' W97,23.432'

APPENDIX II

Table 1. Tick capture totals for Mitch Park (residential) by habitat and tick species.

	D. variabilis	*I. scapularis*	*A. maculatum*	*A. americanum*
Woodland (Dry Ice)	32	5	15	1429
Grassland (Dry Ice)	6	5	29	15
Drag	8	39	13	4
TOTAL	**46**	**49**	**57**	**1448**

Table 2. Tick capture totals for Edmond Park (non-residential) by habitat and tick species.

	D. variabilis	*I. scapularis*	*A. maculatum*	*A. americanum*
Woodland (Dry Ice)	9	1	1	795
Grassland (Dry Ice)	1	1	3	7
Drag	14	13	0	5
TOTAL	**24**	**15**	**4**	**807**