UPDATED ECOLOGY AND DISTRIBUTION OF TICK SPECIES IN OKLAHOMA

By

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UPDATED ECOLOGY AND DISTRIBUTION OF

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Abstract: Ticks are known vectors of many zoonotic diseases that affect humans and livestock. The incidence of tick-borne disease continues to increase in humans and companion animals in the U.S. yet distribution maps for several tick vectors in Oklahoma are not available or outdated. Additionally, there is knowledge gap regarding large mammal species which transport ticks as well as how ecoregion affects tick encounter risk for humans and companion animals. To address these knowledge gaps, county-scale tick records from peer-reviewed literature and passive collections were reviewed for Oklahoma as well as active collections. By these methods *D. variabilis*, *D. albipictus*, *I. scapularis*, and *A. maculatum* were identified in 88%, 45%, 66.2%, and 64.9% counties in Oklahoma, respectively. The American black bear (*Ursus americanus*) recolonized into the state of Oklahoma in the mid-1980s. The habitat for black bear is also important for several tick species in Oklahoma which are also known vectors for zoonotic diseases. Currently, there is no information on the tick species parasitizing the black bear in Oklahoma. Between May and August 2014, working with ODWC and NREM, 1159 ticks were collected from 62 bear. Between February and March 13 adult bear and 22 cubs in dens were monitored to determine which tick species fed on them. The primary species found on the bear was *A. americanum* (69.3%) and only 3 ticks were found on adult bears during denning season. Oklahoma has one of the highest annual incidence rates for tick-borne diseases in the U.S. Oklahoma state parks are well-visited creating an opportunity for the public to come into contact with tick-borne diseases. Between April-August 2015, 1035 ticks were systematically collected from six state parks across Oklahoma. There was a higher risk for encountering *A. americanum*, especially in the eastern side of the state. The ticks were tested by PCR for tick-borne pathogens and 9.49% *A. americanum* pools tested positive for *E. chaffeensis*. The data discussed within this thesis provides valuable update concerning the distribution, host selection, and tick encounter rates within the state of Oklahoma.

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CHAPTER I

INTRODUCTION

Ticks are well known vectors of many zoonotic diseases that affect humans and livestock (Mullen & Durden 2009). These tick-transmitted diseases are caused by pathogens including protozoa, bacteria, and viruses (Mullen & Durden 2009). Even without a pathogen present, a tick bite can cause serious allergic and/or toxic reactions, temporary paralysis, and even the wounds caused by a tick bite may produce secondary infections that can be dangerous to humans as well as reducing the value of livestock (ruined hides, gotch ear) (Mullen & Durden 2009; Edwards et al. 2011). Because of this, ticks are an important vector to study in the fields of public health and veterinary health.

When evaluating the risks of tick-borne diseases within a given area or state, it is important to know where the tick species are distributed before most ecologically-relevant questions can be asked. Oklahoma tick species of medical and veterinary health concern have different distributions within the eleven ecoregions throughout the state. The distribution of these tick species reported in the literature is outdated and surveys are needed to update these distributions. These county-level surveys would provide a better understanding of tick-borne diseases from an epidemiological standpoint.

In addition to knowing where specific tick species are distributed within a given area, ticks are unable to move great distances on their own and require the movement of their host to transport them from location to location. Mainly, ticks are transported by

White-tailed deer (*Odocoileus virginianus)* as the deer move throughout their home ranges in the state (Childs & Paddock 2003). Other tick transporters in the southcentral United States include coyotes and feral hogs (Bloemer et al. 1988; Sanders et al. 2013). In order to better understand how ticks move throughout the state, more research is needed to focus on Oklahoma's larger mammals that could also be used as transient hosts.

As ticks use their hosts to move in a given region, they potentially move throughout the state as vectors of one or more tick-borne pathogens. This creates a potential risk to humans, pets, and livestock that may come within the range of tick hosts and thus come into contact with the ticks. Recreational areas, such as state parks, are areas of special concern as the habitat is usually left for conservation purposes and may leave park visitors and employees at risk for tick-borne pathogens. Thus, studies in state parks can provide valuable information regarding the risk of exposure to ticks and tick borne pathogens in the wider ecoregion (Eisen et al. 2013).

This thesis addresses the gaps in knowledge concerning the ecology and distribution of these ticks within the State of Oklahoma. The research objectives for the research described in this thesis are:

1. Update the distribution maps of four tick species of medical and veterinary importance within the state at the county level (*Dermacentor variabilis*, *Dermacentor albipictus*, *Ixodes scapularis* and *Amblyomma maculatum*).
2. Investigate the possibility of Oklahoma's population of American black bear (*Ursus americanus*) as tick hosts and transporters within a given region.

3. Investigate the risk of exposure to ticks and tick borne pathogens within six different state parks that are in six different ecoregions within the state.

CHAPTER II

REVIEW OF LITERATURE

Ticks are exclusively parasitic animals from the Order Parasitiformes, Suborder Ixodida (Varela-Stokes et al. 2009). The Suborder Ixodida includes three families, Ixodidae, Argasidae, and Nuttalliellidae. The tick species of focus in this thesis are all from the family Ixodidae, also known as the 'Hard Ticks'. The term "hard" refers to the presence of the dorsal shield or scutum in the Ixodidae family (Varela-Stokes et al. 2009). Within the family Ixodidae, there are several genera with the largest being the genus Ixodes.

Hard ticks display sexual dimorphism with the females are capable of expanding to a size larger than the males during blood-feeding as the males either fed in small quantities or not at all, depending on the species (Varela-Stokes et al. 2009). Hard ticks have three feeding stages in their life cycle (larvae, nymph, and adult) (Figure 2.1). In most cases, each feeding stage has a different host. Tick species that exhibit a feeding behavior of three separate hosts are called "three-host" ticks (Varela-Stokes et al. 2009). Most hard tick species mate on the host. After the female fully engorges, she will drop off the host, find a suitable habitat and lay between 2,000-18,000 eggs and die (Varela-Stokes et al. 2009).

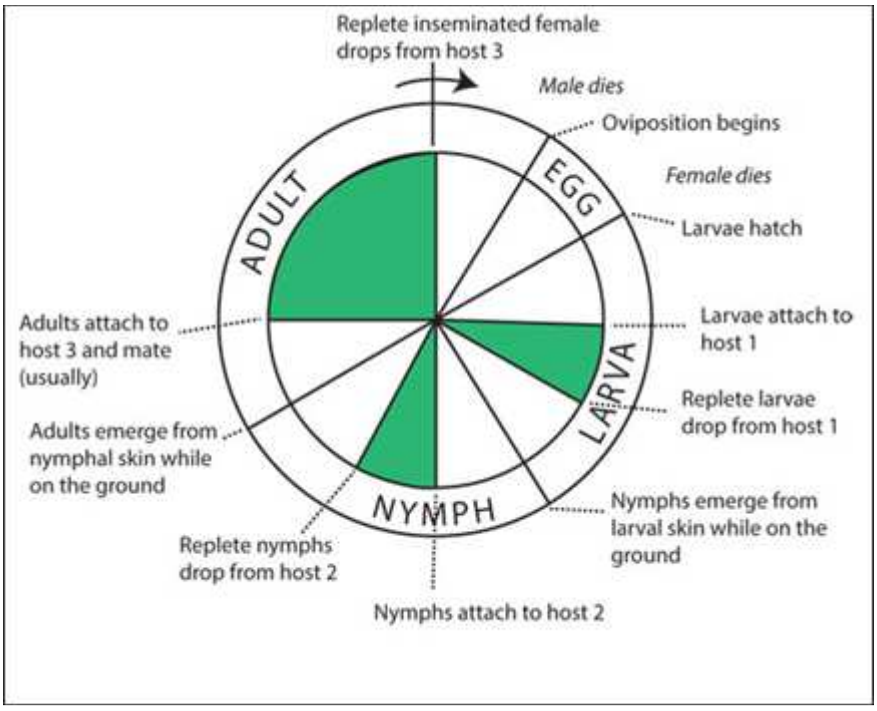

Figure 2.1. Three-Host tick life cycle. Photo credit: University of Missouri Extension

Many species of hard ticks display a host-seeking behavior called "questing". During a particular time of the day, the tick will climb the vegetation and extend the forelegs while waiting for a host to pass (Figure 2.2) (Varela-Stokes et al. 2009). The tick's activity to find a bloodmeal will depend on temperature and humidity due to being sensitive to desiccation (Semtner & Hair 1973). Some hard ticks will also "hunt" for a host, traveling short distances towards any host cues, such as CO_2 (Varela-Stokes et al. 2009).

Figure 2.2. An example of a male Lone Star tick questing on vegetation.

Hard tick species are generally non-nidicolous and are often found in wooded habitats, areas of secondary growth, and/or disturbed areas that contain their host species (Varela-Stokes et al. 2009). Being non-nidicolous, hard tick species actively pursue their hosts or wait for the host (questing) to brush past them. Nidicolous tick species live and develop in nests, feeding often on their hosts.

Environmental factors play a significant role in the tick's ability to transmit pathogens. Due to their sensitivity to desiccation, preferred tick habitats contain shelter from high temperatures and low humidity, such as dense leaf litter (Varela-Stokes et al. 2009). Ticks are very susceptible to desiccation and, as such, need to be in thick vegetation which generally has higher humidity. Additionally, the microclimate also affects the height on which the ticks crawl up the vegetation and wait for their next bloodmeal (Randolph 2004). In hot dry climates, ticks will quest lower in the vegetation, often coming in contact with only small animals, or none at all (Randolph 2004).

Humidity plays a major role as an environmental barrier to tick presence and activity, being critical in their resting and feeding behaviors. Ticks may go months or even years without a bloodmeal so they must be able to maintain their water/ion balance

(Openchain & Galun 1982). To achieve this balance, ticks have developed many physical, morphological, and behavioral characteristics to counter their loss of water. When they sense an increase in humidity, the tick crawls out of their resting habitat and into the vegetation to quest for a bloodmeal. When the tick senses a decrease in humidity or a change in water balance, it climbs back down into the substrate where the humidity is higher and it can re-equilibrate the water/ion balance again. Should the tick not be able find an adequate place to re-hydrate, it will dehydrate and die (Openchain & Galun 1982).

Each major tick species in Oklahoma has very specific characteristics which make them unique as well as highly adapted to the hosts they feed on as well as the habitats in which they live. The next section of the literature review will highlight specific aspects of each tick species that are common in Oklahoma.

1) *Dermacentor variabilis* **(American Dog Tick):**

Figure 2.3. *Dermacentor variabilis*. Photo credit: Dr. R. Grantham, Oklahoma State University

Dermacentor variabilis (The American Dog tick) (Fig 2.3) is distributed throughout most of the central and eastern United States (Fig 2.4) as well as most of Oklahoma (Fig 2.5) (Eddy 1940). This species is the principle vector of *Rickettsia*

rickettsii (pathogen responsible for Rocky Mountain spotted fever) (Dergousoff et al. 2013) as well as *Francisella tularensis*, the causative agent of Tularemia (Dergousoff et al. 2013).

Adult *D. variabilis* are normally found on a wide variety of small to medium sized mammals (Bishopp et al. 1945) with rodents being the preferred host for the immature stages (Bishopp et al. 1945). This species of tick is found in a wide variety of habitats. Bishopp et al. (1945) reported finding these ticks more in grassy areas where as Sonenshine & Levy (1972) found a higher frequency of adult ticks captured in a mixed upland and hickory dominant forest areas. Peak abundance varies between locations (Burg 2001). Some areas report peaks between late May to late June, some mid-July, and some late June to or early July (Sonenshine 1972).

Figure 2.4. CDC range map for *D. variabilis*. Credit: CDC online.

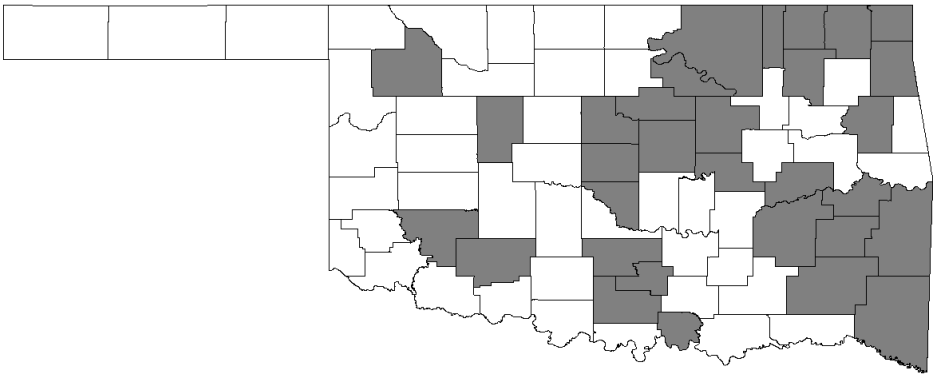

Figure 2.5. Historical populations of *D. variabilis* within the state of Oklahoma. Recreated map from Master Thesis (Eddy 1940).

2) *Dermacentor albipictus* **(Winter Tick)**:

Figure 2.6 *Dermacentor albipictus*. Photo credit: University of GA

Dermacentor albipictus (Winter tick) (Fig 2.6) has a reported range in the subarctic regions of North American (Baldridge et al. 2009). It also has been recorded in Oklahoma since 1940 (Eddy 1940). Interestingly, the distribution of this species has been reported within the range of white-tailed deer (*Odocoileus virginianus*) especially in the southern states of the U.S. (Baldridge et al. 2009). Known as a vector of *Anaplasma marginale, Anaplasma phagocytophilum* and *Francisella*-like endosymbionts (Baldridge

et al. 2009), it has not been identified as a vector for *Borrelia burgdorferi* even those this pathogen was detected in ticks collected in Oklahoma (Kocan et al. 1992).

This species is a one host tick that primarily parasitizes Cervids but will also infest cattle (Baldridge et al. 2009). This species appears to have benefited from the substantial deer population recovery in the central United States (Cortinas & Kitron 2006). In Missouri, a higher prevalence of *D. albipictus* was reported on deer in the upland habitats rather than the lowland habitats (Kollars et al. 1997). In Alberta, Canada, engorged female *D. albipictus* drop off their hosts and lay eggs in June with the eggs hatching between August and September (Drew et al. 1985). The larvae molt into nymphs and adults during the winter and early spring (Drew & Samuel 1985). There are, however, no reports of the phenology of this tick species in the southern United States.

3) *Ixodes scapularis* **(Blacklegged Tick):**

Figure 2.7. *I. scapularis*. Photo credit: Dr. R. Grantham, Oklahoma State University

Ixodes scapularis (The blacklegged tick) (Fig 2.7) has a reported range throughout the central and eastern United States (Fig 2.8) (Dennis et al. 1998). *Ixodes scapularis* is also present in Oklahoma but the distribution of the tick within the state is based on old data which needs to be updated (Fig 2.9) (Dennis et al. 1998).

Ixodes scapularis is primarily known as most important tick species of public health importance in North America because it is the main vector for the agent of Lyme's disease (*Borrelia burgdorferi*), Human Granulocytic Anaplasmosis (HGA) (*Anaplasma phagocytophilum*), Babesiosis (*Babesia microti*) (Hutchinson et al. 2015) as well as other bacteria (Yabsley et al. 2009; Jin et al. 2012; Johnson et al. 2015).

The hosts for the larvae and nymphs include small mammals and lizards/skinks (Durden et al. 2002, Garvin et al. 2015) while the reported hosts for adults are mainly cattle and white-tailed deer (Barnard 1981, Kocan et al. 1992; Schmidtmann et al. 1998). Bear may also be a possible host for this tick species as was reported in Georgia, Florida and Louisiana (Yabsley et al. 2009; Leydet Jr. & Liang 2013).

Ixodes scapularis is normally associated with forested ecoregions (Dennis et al. 1998). In southeast Oklahoma, adult female Blacklegged ticks were present on cattle from October through April and adult males between October and January (Barnard 1981). In the southern US, the peak abundance for adult females is normally from late October into December with a small peak again in March and April (Barnard 1981). Older literature reports that immatures feed on their hosts between June and December and peak abundance of nymphs were observed in October (Barnard 1981).

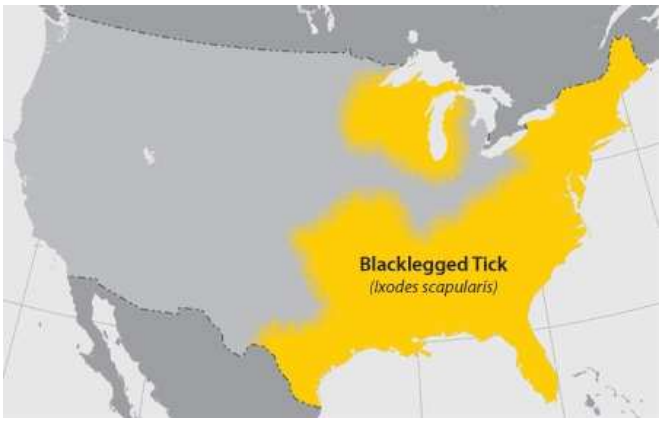

Figure 2.8. CDC range map for I. scapularis. Credit: CDC online.

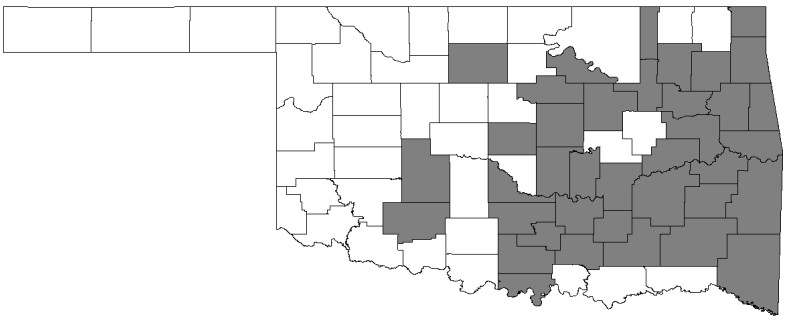

Figure 2.9. Historical populations of *I. scapularis* within the state of Oklahoma. Recreated from Dennis et al. 1998.

4) *Amblyomma maculatum* **(Gulf Coast Tick):**

Figure 2.10. *A. maculatum*. Photo credit: Dr. R. Grantham, Oklahoma State University

Amblyomma maculatum (the Gulf Coast Tick) (Fig 2.10) is a tropical species of tick, once found along the Gulf Coast of Southern U.S. states, now found in Oklahoma (Fig 2.11) (Barker et al. 2004). The earliest report of the Gulf Coast tick in Oklahoma was 1942 in Pittsburg and Comanche Counties (Barker et al. 2004). The introduction of the Gulf Coast tick into Oklahoma is attributed to cattle being brought into Oklahoma from Gulf Coast regions (Teel et al. 2010). The population distribution of this tick species

throughout the state is based on surveys carried out 20 years ago and updated information is required (Fig 2.12) (Barker et al. 2004).

Amblyomma maculatum is an important vector of pathogens which affect both humans and companion animals. *Rickettsia parkeri*, a milder form of Rocky Mountain spotted fever, was first identified in Gulf Coast ticks in 1937. Since then, this pathogen of public health importance has been reported throughout the Southern US (Trout et al. 2010). Although one nymph in Oklahoma was reported to be infected with *R. parkeri* (Sumner et al. 2007), this pathogen has not been found in any other samples from the state (Paddock et al. 2015). The Gulf Coast tick is also an important vector for *Hepatozoon americanum*, an important pathogen which affects canine populations in the southern US (Ewing & Panciera 2003).

The *A. maculatum* tick is a three-host tick and has been collected from 71 species of birds and mammals in the U.S. (Teel et al. 2010). Larvae have been recorded from 38 species and nymphs from 44 (overlapping species) (Teel et al. 2010). The Gulf Coast tick has been collected from cattle, horses, deer, coyote and dogs (in Oklahoma) (Barker et al. 2004) and bear in Georgia and Florida (Yabsley et al. 2009). These ticks appear to have an attachment preference on the inner surface of the ears for cattle and White-tailed deer (Schmidtmann et al. 1998).

The habitat types that are suitable for Gulf Coast ticks in Oklahoma include the grand prairie, west cross timbers, cross timbers, Cherokee prairies, central rolling red plains and prairies, and grasslands dominated by big bluestem, little bluestem, switchgrass, and yellow Indian grass are bordered by wooded uplands of chiefly post oak and blackjack oak (Teel et al. 2010).

In the northern counties of Oklahoma, peak abundance of *A. maculatum* larvae and nymphs (on eastern meadowlarks) has been reported in July and August respectively (Teel et al. 2010). Adults have been most abundant on cattle in the April to June (Teel et al. 2010). There is a current need to confirm this phenology pattern after climate changes and distribution changes of the tick within the state in the last 20 years.

Figure 2.11. CDC range map for *A. maculatum*. Credit: CDC online.

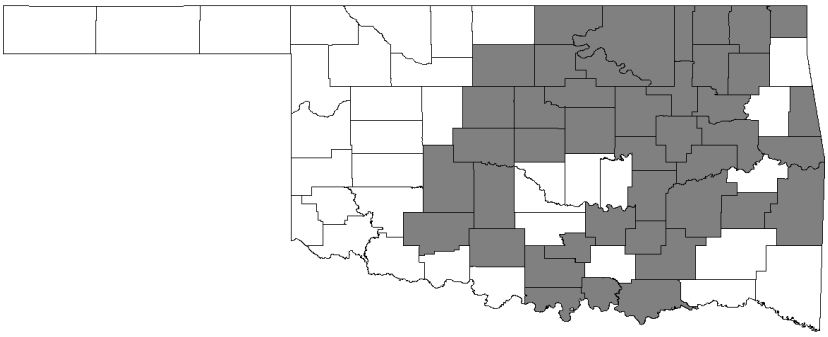

Figure 2.12. Historical populations of *A. maculatum* within the state of Oklahoma. Recreated from Barker et al. 2004.

5) *Amblyomma americanum* **(Lone Star Tick):**

Figure 2.13. *A. americanum*. Photo credit: Dr. R. Grantham, Oklahoma State University

Amblyomma americanum (Lone Star Tick) (Fig 2.13) has been historically present in the Southern part of the US and has been slowly invading new areas during the last 30 years. It is now found up into Maryland and Nebraska (Figure 2.14). Lone star ticks have historically been associated with the Southeastern portion of Oklahoma (Fig 2.15) (Semtner & Hair 1973) and have now been described throughout almost the entire state (Barrett et. al. 2015).

Next to *I. scapularis*, this tick species is one of the most important tick vectors of pathogens affecting humans and companion animals in the United States (Childs & Paddock 2003). It is has been reported to transmit pathogens such as *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Francisella tularensis*, and *Borrelia lonestari*, some *Rickettsia spp*. and *Cytauxzoon felis* (Mullen & Durden 2009; Springer et al. 2014). It is also the vector the recently described Heartland Virus which claimed its 3rd victim in the United States in Eastern Oklahoma in the summer of 2014 (Savage et al. 2013; Press 2015). There is also a possibility that it is the vector species for Bourbon Virus, a second tick-borne virus recently described in Missouri with the 2nd case occurring in Payne County, Oklahoma in May 2015 (Kosoy et al. 2015).

A notorious tick pest for humans and animals in the U.S., all three stages of this species are opportunistic in terms of host selection (Mullen et al. 2009). It would appear that any mammal or ground feeding bird have potential to be a host for this species, as they are aggressive and actively host-seek over wide areas. White-tailed deer are considered the primary host and transporters of adults (Bloemer et al.1988; Mullen & Durden 2009). All stages of tick have been recorded all over the body of deer but adults seem to concentrate on the outer ear, head, udder, and escutcheon whereas the nymphs stick to the ears (both inner and outer) and the larvae have been found on the ears and forelegs (Bloemer et al. 1988).

In Oklahoma, adult ticks can be found on the ground from February into early April and can be found questing on vegetation from late April to August (Semtner & Hair 1973). The peak abundance for adult species has been recorded to be in late May or early June (Semtner & Hair 1973). Adults collected in Cherokee Co. OK were more likely to be found in either a Persimmon-sassafras grove or in a winged elm wood. However, the highest number of ticks were found in Postoak-Blackjack Oaks (Semtner et al. 1971).

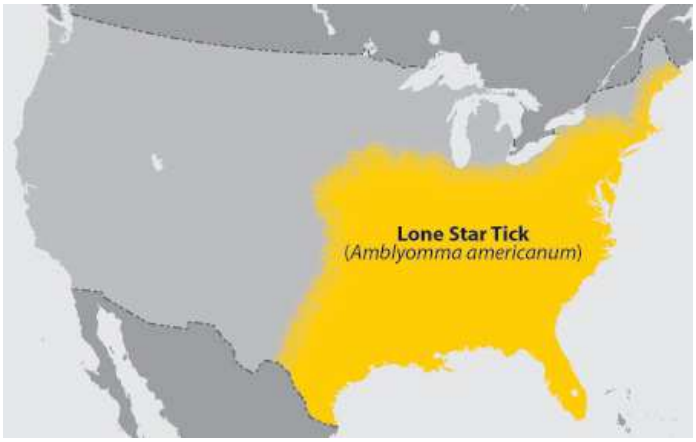

Figure 2.14. CDC range map for *A. americanum*. Credit: CDC online.

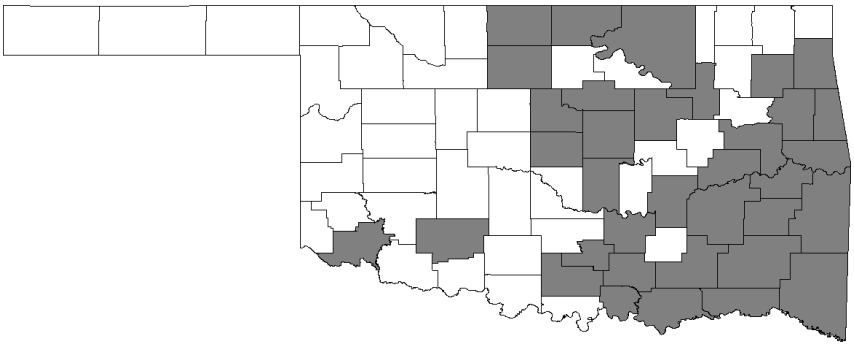

Figure 2.15. Historical populations of *A. americanum* within the state of Oklahoma. Recreated from Springer et al. 2014.

One of the first components in understanding vector borne diseases within a given region is to know where specific tick vectors are distributed within that area. As has been documented in Figures 2.5, 2.9, 2.12 and 2.15, no surveillance of four of these tick species has been actively carried out for almost 20 years. There is no published record of the distribution of *D. albipictus* in the state. It is important then to know where tick vectors are present as questions develop regarding the epidemiology and ecology of each tick-borne disease within Oklahoma and the wider Great Plains region.

Known and possible host of tick species and their presence in Oklahoma

Ticks do not travel far within a given area and rely on their hosts to take them from one place to another (Ruiz-Fons & Gilbert 2010). In fact, this movement normally occurs during the act of feeding so the movement of ticks has nothing to do with their own need to change environments but is an effect of the their ectoparasitic lifestyle.

The white-tailed deer (*Odocoileus virginianus*) is a keystone species in its habitat and is a critical host for most important tick species in the US and particularly in

Oklahoma for the five species summarized above (Bishopp et al. 1945; Bloemer et al. 1988; Kocan et al. 1992; Schmidtmann et al. 1998; Waller & Alverson 1997). As ticks do not move far on their own, deer are considered play a major role in the distribution of these tick species (Childs & Paddock 2003). Adult ticks for each of these five species have also been identified in the Southcentral regions of the US on coyotes (Bloemer et al. 1988) and feral hogs (Sanders et al. 2013) which also serve to move them throughout a given area.

Another important transporter of ticks within their home ranges are American black bear (*Ursus americanus*) (Yabsley et al. 2009). The American black bear has a range that incorporates most of the North American continent, down to the northern ranges of Mexico (nwf.org, 2015). Depending on the abundance of food available, annual home ranges have been recorded as large as 116km^2 for adult males and 12km^2 for adult females (Smith & Pelton 1990). Researchers have observed that black bears are more active during the summer months, which is during the black bear breeding season (Garshelis & Pelton 1980). When food is abundant, black bears can become large with males reaching 600lbs and females reaching 200-300lbs (nwf.org, 2015, personal observation). This species is able to live up to 30 years in the wild (nwf.org, 2015) which makes the black bear a potentially long lived, large mammal with extensive home ranges which may increase the possibility of being an important host and distributor for many tick species (Zolnik et al. 2015). Black bear have been associated with various tick species found in Oklahoma. Nims & Durden (2011) identified *I. scapularis* on black bear in northern Georgia while Yabsley et al. (2009) identified *A. americanum*, *D. variabilis, I. scapularis* and *A. maculatum* on black bear sampled in southern Georgia and northern

Florida. These same four species were identified from black bear in Louisiana by Leydet and Liang (2013) while Manville (1978) identified *Dermacentor variabilis*, *D. albipictus* and *Ixodes scapularis* on free-ranging bear in Wisconsin.

The American black bear (*Ursus americanus*) is an important mega omnivore that was once found all over the state of Oklahoma (Lyda & Helgren 2007). Recolonization efforts in Arkansas in the 1960s have proved to be successful and it is estimated that the black bear returned to Oklahoma in the 1980s (Lyda & Helgren 2007). Oklahoma black bears inhabit a variety of habitats including oak-hickory and oak-pine forests (Lyda & Helgren 2007) which are the same habitats utilized by some of Oklahoma's tick species. Bears may be important hosts to these ticks and they may be spreading ticks throughout their home range, bringing ticks into contact with other wildlife, livestock, humans and/or pets. To date, there is no information published on the tick species that parasitize Oklahoma black bears.

The ecology of tick species and tick-borne pathogens in State Park systems.

When considering the ecology of tick species and the pathogens they transmit, it is often difficult to find smaller representative study areas in which to develop ideas and models of what is happening on a wider scale. In the United States, there is a system of National Parks as well as system of State Parks. These State Parks are developed wilderness areas, normally centralized around a particular recreational sites (lake, river, and mountain) and the environment is purposely left as pristine and undeveloped as possible (Eisen et al. 2013). It is within these park ecosystems that relationships can begin to be understood between various habitats, ticks species, their varied hosts, and the

possible pathogens that may be transmitted to humans and companion animals which come to enjoy the quieting effects of a wilderness area.

Much of our understanding of ticks and their environments have come from studies centered in state and local parks throughout the United States (Eisen et al. 2013). For example:

1) Researchers in Wisconsin surveyed four state parks for the presence of *I. scapularis* between May and June 1998 using a dragging sampling method along hiking trails. They determined the average risk of encountering an infected nymph (with *Borrelia burgdorferi*) ranged from 0.5 to 5.2 infected nymphs per hour (Paskewitz et al. 2001).
2) Researchers surveyed for the presence of ticks in eight parks in Texas between 1990 and 1992. They reported finding *Borrelia* spirochetes in 1.03% of the 5,141 *A. americanum* collected (Rawlings & Teltow 1994).
3) A study in California investigated both hiking trails and picnic areas within a Regional park and found three species of human-biting ticks. *Borrelia* spirochetes were identified in 1.6% of the *D. variabilis* and 0.6% of the *I. pacificus* collected (Lane 1996).
4) On the eastern side of United States, researchers in Florida conducted a 2 year study in a state park and recreational area and found four major human-biting ticks: *I. scapularis*, *A. americanum*, *A. maculatum*, and *D. variabilis* (Cilek & Olson 2000). They reported that the ticks were collected seasonally and the greatest risk for tick attachment was at their campsites where almost 60% of adult and nymphal ticks were collected (Cilek & Olson 2000).

5) Finally, though by no means all, a recent study from a National Military Park in Pennsylvania reported *I. scapularis* with a *Borrelia burgdorferi* infection risk index of 1.3 infected nymphs/hour (Han et al. 2014).

As Lyme disease is a serious disease, it is noteworthy that the majority of park studies focus on ticks that are vectors or potentially could be vectors of *B. burgdorferi*. However, there are other serious tick-borne pathogens that need to be investigated as well.

Some of the critical aspects which can be gleaned from working on ticks and their associated pathogens in state park system include (modified from Eisen et al. 2013):

1) The risk for exposure to vector-borne pathogens in U.S. national and state parks is poorly understood.
2) Active tick surveillance in a state park system, if complemented by risk modeling, could assist in identifying where and when certain park personnel and visitors are at greatest risk for exposure to vectors and vector-borne pathogens.
3) This improved knowledge base then, at a local level, could lead to the development of detailed information (high risk areas and activity time periods) which could be disseminated to park visitors and specifically crafted to inform park personnel about the risks associated with tick vectors and vector-borne pathogens and provide practical suggestions for personal protection measures.
4) These aspects learned within these state park systems would then be directly applicable to a wider geographic context within the United States as well as worldwide.

The Oklahoma state park system is particularly well-used, ranking 15th in the U.S. in annual visitation (Siikamaki 2011; Siderelis et al. 2012). Most popular recreation

activities in Oklahoma state parks, including hiking, camping, and dog-walking, are associated with a high risk of exposure to ticks and tick-borne diseases. Of particular concern for public health officials in Oklahoma is: (1) the temporal overlap between the spring and summer peak of recreation in parks and the period during which ticks are most active, (2) Oklahoma's high incidence rates for several tick-borne diseases, including Rocky Mountain Spotted Fever, Ehrlichiosis, Tularemia (*Francisella tularensis*), and Southern Tick Associated Rash illness [STARi] (Department of Health (OSDH) 2014) and (3) the possible presence of other tick-borne diseases that cause substantial morbidity in other regions but have uncertain distribution and incidence in Oklahoma. By conducting active surveillance in state parks across Oklahoma during the summer months, we will begin to develop a better understanding of the ecology of tick species and assess the risk of exposure to specific pathogens.

REFERENCES CITED

Allan, B. F., L. S. Goessling, G. A. Storch, R. E. Thach. 2010. Blood Meal Analysis to Identify Reservoir Hosts for *Amblyomma americanum* Ticks. Emerging Infectious Diseases. **16**(3): 433-440

Baldridge, G. D., G. A. Scoles, N. Y. Burkhardt, B. Schloeder, T. J. Kurtti, U. G. Munderloh. 2009. Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). J. Med. Entomol. **46**(3): 625-632

Bales, S. L., E. C. Hellgren, D. M. Leslie Jr., J. Hemphill Jr. 2005. Dynamics of a recolonizing population of black bears in the Ouachita Mountains of Oklahoma. Wildlife Society Bulletin. **33**(4):1342-1351

Barker, R. W., A. A. Kocan, S. A. Ewing, R. P. Wettemann, M. E. Payton. 2004. Occurrence of the Gulf Coast Tick (Acari: Ixodidae) on Wild and Domestic Mammals in North-Central Oklahoma. J. Med. Entomol. **41**(2):170-178

Barnard, D. R. 1981. Seasonal Activity and Preferred Attachment Sites of *Ixodes scapularis* (Acari: Ixodidae) on Cattle in Southeastern Oklahoma. Journal of the Kansas Entomological Society. **54**(3):547-552

Barrett, A W., B. H. Noden, J. M. Gruntmeir, T. Holland, J. R. Mitcham, J. E. Martin, E. M. Johnson, S. E. Little. 2015. County Scale Distribution of *Amblyomma americanum* (Ixodida: Ixodidae) in Oklahoma: Addressing Local Deficits in Tick Maps Based on Passive Reporting. J. Med. Entomol. **52**: 269-273

Bertrand, M. R., M. L. Wilson. 1996. Microclimate-Dependent Survival of Unfed Adult *Ixodes scapularis* (Acari: Ixodidae) in Nature: Life Cycle and Study Design Implications. J. Med. Entomol. **33**(4): 619-627

Bishopp, F. C., H. L. Trembley. 1945. Distribution and Hosts of Certain North American Ticks. The Journal of Parasitology. **31**(1): 1-54

Bloemer, S. R., R. H. Zimmerman, K. Fairbanks. 1988. Abundance, Attachment Sites, and Density Estimators of Lone Star Ticks (Acari: Ixodidae) Infesting White-Tailed Deer. J. Med. Entomol. **25**(4): 295-300

Burg, J. G. 2001. Seasonal activity and spatial distribution of host-seeking adults of the tick *Dermacentor variabilis*. Medical and Veterinary Entomology. **15**: 413-421

Childs, J. E. and C. D. Paddock. 2003. The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. Annu. Rev. Entomol. **48**: 307-337

Cilek, J. E. & M. A. Olson. 2000. Seasonal distribution and abundance of ticks (Acari: Ixodidae) in Northwestern Florida. J. Med. Entomol. **37**(3): 439-444

Cortinas, M. R., U. Kitron. 2006. County-level surveillance of white-tailed deer infestation by *Ixodes scapularis* and *Dermacentor albipictus* (Acari: Ixodidae) along the Illinois River. J. Med. Entomol. **43**(5): 810-819

Dennis, D. T., T. S. Nekomoto, J. C. Victor, W. S. Paul, J. Piesman. 1998. Reported Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the United States. J. Med. Entomol. **35**(5):629-638

Department of Health (OSDH). 2014. Tickborne and Mosquitoborne Disease Prevention. Online: http://www.ok.gov/health/Disease,_Prevention,_Preparedness/Acute_Disease_Ser

vice/Disease_Information/Tickborne_and_Mosquitoborne_Diseases/index.html (Accessed 2-23-14).

Dergousoff, S. J., T. D. Galloway, L. R. Lindsay, P. S. Curry, N. B. Chilton. 2013. Range Expansion of *Dermacentor variabilis* and *Dermacentor andersoni* (Acari: Ixodidae) Near Their Northern Distributional Limits. Journal of Medical Entomology. **50**(3) 510-520

Drew, M. L., W. M. Samuel. 1985. Factors affecting transmission of larval winter ticks, *Dermacentor albipictus* (Packard), to moose, *Alces alces* L., in Alberta, Canada. Journal of Wildlife Diseases. **21**(3): 274-282

Drew, M. L., W. M. Samuel, G. M. Lukiwski, J. N. Willman. 1985. An evaluation of burning for control of winter ticks, *Dermacentor albipictus*, in central Alberta. Journal of Wildlife Diseases. **21**(3): 313-315

Durden, L.A., J. H. Oliver Jr., C. W. Banks, G. N. Vogel. 2002. Parasitism of lizards by immature stages of the blacklegged tick, *Ixodes scapularis* (Acari, Ixodidae). Experimental and Applied Acarology. **26**:257-266

Eddy, G. W. 1940. The Ticks of Oklahoma. Master's thesis. Oklahoma Agricultural and Mechanical College

Edwards, K. T., J. Goddard, T. L. Jones, C. D. Paddock, A. S. Varela-Stokes. 2011. Cattle and the Natural History of *Rickettsia parkeri* in Mississippi. Vector-Borne and Zoonotic Diseases. **11**(5):485-491

Eisen, L, D. Wong, V. Shelus, R. J. Eisen. 2013. What is the risk for exposure to vector-borne pathogens in United States National parks? J. Med. Entomol. **50**(2): 221-230

Ewing, S. A. and R. J. Panciera. 2003. American canine Hepatozoonosis. Clin. Microbiol. Rev. **16**(4): 688-697

Ewing, S. A., R. J. Panciera, K. M. Kocan, N. L. Ge, R. D. Welsh, R. W. Olson, R. W. Barker, L. E. Rice. 1997. A winter outbreak of anaplasmosis in a nonendemic area of Oklahoma: a possible role for *Dermacentor albipictus*. J. Vet. Diagn. Invest. **9**: 206-208

Garshelis, D. L & M. R. Pelton. 1980. Activity of black bears in the Great Smoky Mountains National Park. Journal of Mammalogy. **61**(1): 8-19

Garvin, S. D., B. H. Noden, J. W. Dillwith, S. F. Fox, M. E. Payton, R. W. Barker. 2015. Sylvatic infestation of Oklahoma reptiles with immature *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. **52**(5): 873-878

Han, G. S., E. Y. Stromdahl, D. Wong, A. C. Weltman. 2014. Exposure to *Borrelia burgdorferi* and other tick-borne pathogens in Gettysburg National Military Park, South-Central Pennsylvania, 2009. Vector-Borne and Zoonotic Diseases. **14**(4): 227-233

Hutchinson, M. L., M. D. Stohecker, T. W. Simmons, A. D. Kyle, M. W., Helwig. 2015. Prevalence rates of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia microti* (Piroplasmida: Babesiidae) in host-seeking *Ixodes scapularis* (Acari: Ixodidae) from Pennsylvania. J. Med. Entomol. **52**(4): 693-698

Jiang, J., Y. Stromdahl, A. L. Richards. 2012. Detection of *Rickettsia parkeri* and *Candidatus Rickettsia andeanae* in *Amblyomma maculatum* Gulf Coast Ticks Collected from Humans in the United States. Vector-Borne and Zoonotic Diseases. **12**(3):175-182

Jin, H., F. Wei., Q. Liu., J. Qian. 2012. Epidemiology and Control of Human Granulocytic Anaplasmosis: A Systemic Review. Vector-Borne and Zoonotic Diseases. **12**(4): 269-274

Johnson, D. K., E. K. Schiffman, J. P. Davis, D. F. Neitzel, L. M. Sloan, W. L. Nicholson, T. R. Fritsche, C. R. Steward, J. E. Ray, T. K. Miller, M. A. Feist, T. S. Uphoff, J. J. Franson, A. L. Livermore, A. K. Deedon, E. S. Theel, B. S. Pritt. 2015. Human infection with Ehrlichia muris-like pathogen, United States, 2007-2013. Emerg. Infect. Dis. **21**(10): 1794-1799

Keirans, J.E., H. J. Hutcheson, L. A. Durden, J. S. H. Klompen. 1996. *Ixodes* (*Ixodes*) *scapularis* (Acari: Ixodidae): Redescription of all Active Stages, Distribution, Hosts, Geographical Variation, and Medical and Veterinary Importance. J. Med. Entomol. **33**(3): 297-318

Kocan, A. A., S. W. Mukolwe, G. L. Murphy, R. W. Barker, K. M. Kocan. 1992. Isolation of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) from *Ixodes scapularis* and *Dermacentor albipictus* Ticks (Acari: Ixodidae) in Oklahoma. J. Med. Entomol. **29**(4):630-633

Kollars, Jr. T. M., L. A. Durden, E. J. Masters, J. H. Oliver, Jr. 1997. Some factors affecting infestations of white-tailed deer by blacklegged ticks and winter ticks (Acari: Ixodidae) in southeastern Missouri. J. Med. Entomol. **34**(3): 372-375

Kollars Jr., T. M., J. H. Oliver Jr., P. G. Kollars, L. A. Durden. 1999. Seasonal Activity and Host Associations of *Ixodes scapularis* (Acari: Ixodidae) in Southeastern Missiouri. J. Med. Entomol. **36**(6): 720-726

Kosoy, O., A. Lambert, D. Hawkinson, D. Pastula, C. Goldsmith, D. Hunt, J. Staples. 2015. Novel Thogotovirus associated with febrile illness and death, United States, 2014. Emerg. Infect. Dis. **12**(5): 760-764

Lane, R. S. 1996. Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in Northern California. Am. J. Trop. Med. Hyg. **55**(2): 165-173

Leydet Jr., B. F., F.T. Liang. 2013. Detection of human bacterial pathogens in ticks collected from Louisiana black bears (*Ursus americanus luteolus*). Ticks and Tick-borne Diseases. **4**: 191-196

Lyda, S. B., E. C. Hellgren. 2007. Diurnal Habitat Selection and Home-range Size of Female Black Bears in the Ouachita Mountains of Oklahoma. Proc. Okla. Acad. Sci. **87**: 55-64

Mackay, A., L. Foil. 2005. Seasonal and geographical distribution of adult *Ixodes scapularis* Say (Acari: Ixodidae) in Louisiana. Journal of Vector Ecology. **30**(2): 168-170

Manville II, A. M. 1978. Ecto-and endoparasites of the black bear in northern Wisconsin. Journal of Wildlife Diseases. **14**(1): 97-101

Mullen, G. R., L. A. Durden. 2009. Medical and Veterinary Entomology. MA: Academic Press. Chapter 26.

Nwf.org. 2015. American Black Bear-National Wildlife Federation. (n.d.). Retrieved November 1, 2015, from http://www.nwf.org/wildlife/wildlife-library/mammals/black-bear.aspx

Nims, T. N. and L. A. Durden. 2011. Ticks and Lice of the Black Bear, *Ursus americanus* Pallas, in Northern Georgia, USA, Including a New State Record for the Chewing Louse, *Trichodectes euarctidos (Phthiraptera: Trichodectidae).* J. Entomol. Sci. **46**(4): 345-347

Openchain, D. & R. Galun. 1982. Physiology of Ticks. Current Themes in Tropical Science. Vol 1. Pergamon Press, Maxwell House, Fairview Park, Elmsford, New York 10523.

Paddock, C. D., A. M. Denison, M. W. Dryden, B. H. Noden, R. R. Lash, S. S. Abdelghani, A. E. Evans, A. R. Kelly, J. A. Hecht, S. E. Karpathy, R. R. Ganta, S. E. Little. 2015. High prevalence of "Candidatus Rickettsia andeanae" and apparent exclusion of *Rickettsia parkeri* in adult *Amblyomma maculatum* (Acari: Ixodidae) from Kansas and Oklahoma. Ticks Tick Borne Dis. **6**(3): 297-302

Paskewitz, S. M., M. Vandermause, E. A. Belongia, J. J. Kazmierczak. 2001. *Ixodes scapularis* (Acari: Ixodidae): Abundance and Rate of Infection with *Borrelia burgdorferi* in Four State Parks in Wisconsin. Journal of Medical Entomology. **38**(1): 33-38

Press, A. (2014, May 28). Rare Heartland Virus kills man in Oklahoma. Retrieved July 25, 2014, from CBS News: http://www.cbsnews.com/news/rare-heartland-virus-kills-man-in-oklahoma/)

Randolph, S. E. 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology. **129**: S37-S65

Rawlings, J. A. & G. J. Teltow. 1994. Prevalence of *Borrelia* (Spirochaetaceae) Spirochetes in Texas ticks. J. Med. Entomol. **31**(2): 297-301

Ruiz-Fons, F. & L. Gilbert. 2010. The role of deer as vehicles to move ticks, Ixodes ricinus, between contrasting habitats. International Journal for Parasitology. **40**(9): 1013-1020

Sanders, D. M., A. L. Schuster, P. W. McCardle, O. F. Strey, T. L. Blankenship, P. D. Teel. 2013. Ixodid ticks associated with feral swine in Texas. J. Vector Ecol. **38**(2): 361-373

Savage, H., M. Godsey Jr., A. Lambert, N. Panella, K. Burkhalter, J. Harmon, R. R. Lash, D. C. Ashley, W. L. Nicholson. 2013. First detection of Heartland Virus (Bunyaviridae: Phlebovirus) from field collected arthropods. Am. J. Trop. Med & Hyg. **89**(3): 445-452

Schmidtmann, E. T., J. F. Carroll, D. W. Watson. 1998. Attachment-Site Patterns of Adult Blacklegged Ticks (Acari: Ixodidae) on White-Tailed Deer and Horses. J. Med. Entomol. **35**(1): 59-63

Semtner, P. J., D. E. Howell, J. A. Hair. 1971. The Ecology and Behavior of the Lone Star Tick (Acarina: Ixodidae) I. The relationship between vegetative habitat type and tick abundance and distribution in Cherokee Co., Oklahoma. Journal of Medical Entomology. **8**(3): 329-335

Semtner, P. J., J. A. Hair. 1973. The Ecology and Behavior of the Lone Star Tick (Acarina: Ixodidae) V. Abundance and seasonal distribution in different habitat types. Journal of Medical Entomology. **10**(6): 618-628

Siderelis, C. R. L. Moore, Y. F. Leung, J. W. Smith. 2012. A nationwide production analysis of state park attendance in the United States. J. Environ. Manag. **99**: 18-26

Siikamaki, J. 2011. Contributions of the US state park system to nature recreation. Proceedings of the National Academy of Sciences USA. **108**: 14031-14036

Smith, T. R. & M. R. Pelton. 1990. Home ranges and movements of black bears in a bottomland hardwood forest in Arkansas. Bears: Their biology and management. **8**: 213-218

Sonenshine, D. E., G. F. Levy. 1972. Ecology of the American Dog Tick, *Dermacentor variabilis*, in a Study Area in Virginia. 2. Distribution in Relation to Vegetative Types. Annals of the Entomological Society of America. **65**(5): 1175-1182

Sonenshine, D. E. 1972. Ecology of the American Dog Tick, *Dermacentor variabilis*, in a Study Area in Virginia. 1. Studies on Population Dynamics Using Radioecological Methods. Annals of the Entomological Society of America. **65**(5): 1164-1175

Springer, Y. P., L. Eisen, L. Beati, A. M. James, R. J. Eisen. 2014. Spatial Distribution of Counties in the Continental United States with Records of Occurrence of *Amblyomma americanum* (Ixodida: Ixodidae). Journal of Medical Entomology. **51**(2): 342-351

Sumner, J. W., L. A., Durden, J. Goddard, E. Y. Stromdahl, K. L. Clark, W. K. Reeves, C. D. Paddock. 2007. Gulf Coast Ticks (*Amblyomma maculatum*) and *Rickettsia parkeri*, United States. Emerging Infectious Diseases. **13**(5):751-753

Teel, P. D., S. W. Hopkins, W. A. Donahue, O. F. Strey. 1998. Population Dynamics of Immature *Amblyomma maculatum* (Acari: Ixodidae) and Other Ectoparasites on Meadowlarks and Northern Bobwhite Quail Resident to the Coastal Prairie of Texas. J. Med. Entomol. **35**(4):483-488

Teel, P. D., H. R. Ketchum, D. E. Mock, R. E. Wright, O. F. Strey. 2010. The Gulf Coast Tick: A Review of the Life History, Ecology, Distribution, and Emergence as an Arthropod of Medical and Veterinary Importance. J. Med. Entomol. **47**(5):707-722

Trout, R., C. D. Steelman, A. L. Szalanski, P. C. Williamson. 2010. Rickettsiae in Gulf Coast Ticks, Arkansas, USA. Emerging Infectious Diseases. **16**(5):830-832

Varela-Stokes, A., J. Goddard, K. T. Edwards, A. Harris, G. Moraru, F. Girao, E. Chenney. 2009. Highlights of Tick-borne Disease Research at Mississippi State University. Journal of the Mississippi Academy of Sciences. **54**(2):131-140

Waller, D. M., W. S. Alverson. 1997. The White-Tailed Deer: A Keystone Herbivore. Wildlife Society Bulletin. **25**(2) 217-226

Yabsley, M. J., T. N. Nims, M. Y. Savage, L. A. Durden. 2009. Ticks and Tick-Borne Pathogens and Putative Symbionts of Black Bears (*Ursus americanus floridanus*) from Georgia and Florida. The Journal of Parasitology. **95**(5):1125-1128

Zolnik, C. P., A. M. Makkay, R. C. Falco, T. J. Daniels. 2015. American black bears as hosts of blacklegged ticks (Acari: Ixodidae) in the Northeastern United States. J. Med. Entomol. DOI: 10.1093/jmc/tjv/092

CHAPTER III

ACTIVE SURVEILLANCE TO UPATE COUNTY SCALE DISTRIBUTION OF FOUR TICK SPECIES OF MEDICAL AND VETERINARY IMPORTANCE OF OKLAHOMA

Abstract

The incidence of tick-borne disease continues to increase in humans and companion animals in the United States yet the distribution maps for several tick vectors in Oklahoma, including *Dermacentor variabilis*, *Dermacentor albipictus*, *Ixodes scapularis* and *Amblyomma maculatum*, are not available or outdated. To address this issue, county-scale tick records from peer-reviewed literature and passive collections were reviewed for Oklahoma. Additionally, dry ice traps, tick drags and harvested deer were utilized to actively collect adult ticks throughout the state. Through these methods, *D. variabilis, D. albipictus, I. scapularis,* and *A. maculatum* were identified in 88% (68/77), 45% (35/77), 66.2% (51/77), and 64.9% (50/77) of counties in Oklahoma, respectively. Baseline maps were developed for the distribution of *D. variabilis* and *D. albipictus* and distribution maps were updated for *I. scapularis* and *A. maculatum*. This data confirms that these four species of ticks continue to be widespread within Oklahoma with a possible recent western expansion of *I. scapularis* populations. It is anticipated that these results will assist efforts to better understand the epidemiology of the different disease caused by pathogens transmitted by these tick species within the Great Plains region.

INTRODUCTION

The incidence of tick-borne disease continues to increase in humans and companion animals in the United States (CDC 2015). One area of concern is the south central region of the US which has seen an increase in ehrlichiosis, Rocky Mountain spotted fever, and tularemia among human populations and ehrlichiosis and hepatozoonosis among companion animals (Openshaw et al. 2010; Dahlgren et al. 2011; Starkey et al. 2013; Little et al. 2014; Carmichael et al. 2014). While apparent that the incidence of these diseases is increasing, the distribution maps for principle tick vectors is this region are either not available, outdated or based on passive collections (Cortinas & Spomer 2014; Springer et al. 2014). By actively monitoring the distribution of a specific tick species, evidence can be gathered that can help explain tick-borne disease patterns within a given area (Jobe et al. 2007; Rand et al. 2007; Hamer et al. 2010; Rydzewski et al. 2012; Lee et al. 2013; Wang et al. 2014). These kinds of studies, also serve as a critical link in educating communities about the risks for possible exposure to various tick-borne pathogens (Bayles & Allen 2013).

The risk of tick-borne pathogen activity in Oklahoma has been described in humans and veterinary species (Openshaw et al. 2010; Dahlgren et al. 2011; Starkey et al. 2013; Little et al. 2014; Carmichael et al. 2014). Public health risk for these infections has particular impact on American Indian communities (Demma et al. 2006; Holman et al. 2009; Folkema et al. 2012). The incidence of Rocky Mountain spotted fever and ehrlichiosis in human populations in Oklahoma has increased dramatically in the past 15 years with the highest incidence occurring in the eastern portion of the state (K. Bradley, state epidemiologist, personal communication). While increased incidence of different

tick-borne diseases is notable, there is relatively little information on the distribution of important tick species which transmit various pathogens in Oklahoma. Currently, no published records exist for distributions of *Dermacentor variabilis* or *Dermacentor albipictus* within the state and currently available distribution maps for *Ixodes scapularis* (Dennis et al. 1998) and *Amblyomma maculatum* (Barker et al. 2004) are based on surveys carried out 10-15 years ago.

Establishing baselines and updating distributions will assist in gaining a better understanding of the various factors involved in the patterns of disease incidence in the state. The purpose of this study was to establish and update distribution maps for four important tick species involved in the transmission of a variety of pathogens within the State of Oklahoma. This study was part of a larger study updating the distribution of the lone star tick in Oklahoma (Barrett et al. 2015). At the outset, it is important to note that the author (J. Mitcham) was involved in the collection of ticks in six of the counties covered by this project.

METHODS AND MATERIALS

Tick species records from Oklahoma. Details of collection methods were published in Barrett et al. (2015). Briefly, in addition to reports summarized from peer-reviewed literature (Table 3.1), which included a Master's thesis from Oklahoma-based institutions, records were compiled from the K.C. Emerson Entomology Museum (Oklahoma State University), which has archived specimens submitted since the 1940s, and from issues of the United States Department of Agriculture's (USDA) Cooperative Economic Insect Reports (CEIR). Due to the lack of a baseline distribution map for *D.*

variabilis, submissions reported on VectorMap, an online database where specimens are linked to places of collection, were included. Updated distribution maps for *I. scapularis* and *A. maculatum* were based on earlier work (Dennis et al. 1998; Barker et al. 2004).

Active Collection of Ticks. Active collection of ticks took place during two seasons: 1) between May and July 2014 in which 50 counties were sampled across Oklahoma and 2) between October and November 2014 in which 8 counties [Beaver Co., Blaine Co., Cleveland Co., Custer Co., Dewey Co., Ellis Co., Grady Co., and Major Co.] were sampled. The summer collection period utilized dry ice trapping, dragging and flagging as the primary collection method (Barrett et al. 2015). Additional active collection efforts during fall 2014 consisted of collecting ticks from harvested deer during Oklahoma Department of Wildlife and Conservation (ODWC) controlled hunts in several Wildlife Management Areas (WMAs). Ticks were collected directly from harvested deer either by study personnel or by ODWC personnel. All ticks were stored in in 70% EtOH and identified in the laboratory using standard keys (Keirans & Litwak 1989; Keirans & Durden 1998).

Data summation. Counties were classified as established or reported for each tick species using criteria described by Dennis et al. (1998). A tick species was considered 1) established in a county if six or more ticks, or more than one life stage was collected or reported at one time period, or 2) reported in a county if less than six ticks, or only one life stage was collected at one time period. Counties described in the USDA-CEIR or museum specimens were considered to have established populations of a specific tick species if enumeration meeting the above standards was met. Distribution

maps for tick species were created using DIVAGIS (Version 7.5.0.0) (http://www.diva-gis.org/).

RESULTS

Dermacentor variabilis*: Dermacentor variabilis* is reported (n=48) or established (n=20) in 88% (68/77) counties in Oklahoma (Figure 3.1). For 50/68 counties, the presence of *D. variabilis* was confirmed through review of peer reviewed publications, museum collections or publicly available records (Table 3.2). Active surveillance during the summer of 2014 added 18 counties to this distribution (Fig. 3.1).

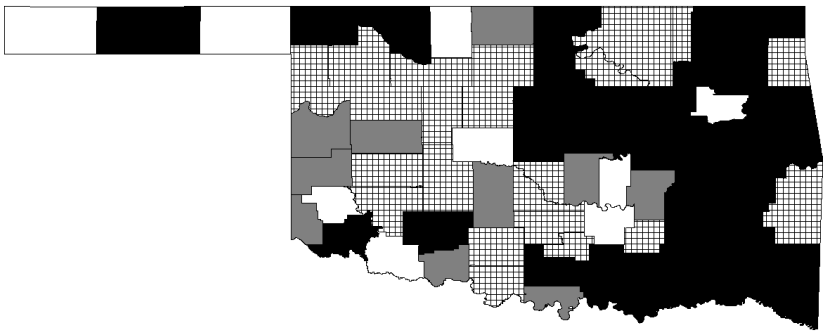

Figure 3.1. Distribution of *D. variabilis* at the county level across the State of Oklahoma. Counties in black = Peer-reviewed, Grey = Active collection, 2014, Crossfill = both methods.

***Dermacentor albipictus*:** *Dermacentor albipictus* is reported (n=20) or established (n=15) in 45.4% (35/77) of the counties in Oklahoma (Figure 3.2). For 32/35 counties, the presence of *D. albipictus* was confirmed through review of peer reviewed publications, museum collections or publicly available records (Table 3.3). Active surveillance in October/November 2014 added 3 counties to this distribution (Figure 3.2).

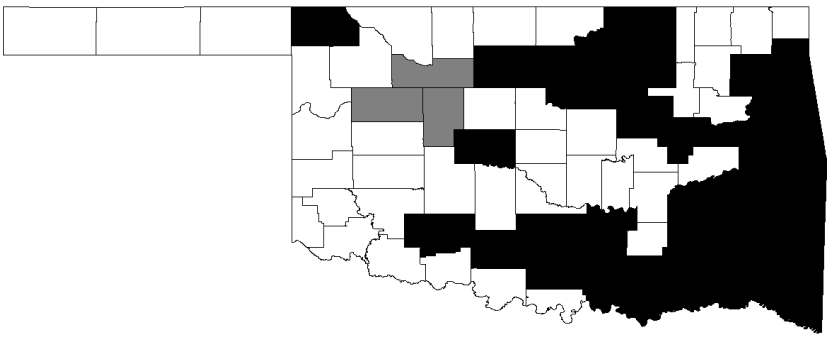

Figure 3.2. Distribution of *D. albipictus* at the county level across the State of Oklahoma. Counties in black = Peer-reviewed, Grey = Active collection, 2014.

Ixodes scapularis: Prior to this study, passive surveillance recorded *I. scapularis* in 61% (47/77) of counties, mainly in the eastern and central portion of the state (Figure 3.3). Active surveillance confirmed *I. scapularis* in 8 counties in summer 2014 and added another 4 counties in fall 2014 to the current distribution (Figure 3.3) (Table 3.4), thus advancing the distribution of this tick further to the west in the state. Overall, *I. scapularis* is currently reported (n=34) or established (n=17) in 66.2% (51/77) counties in Oklahoma.

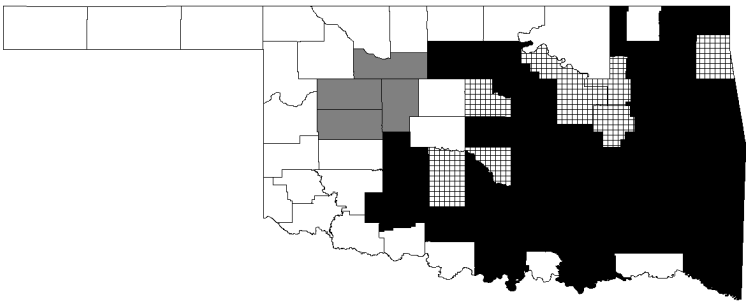

Figure 3.3. Distribution of *I. scapularis* at the county level across the State of Oklahoma. Counties in black = Peer-reviewed, Grey = Active collection, 2014, Crossfill = both methods.

***Amblyomma maculatum*:** Passive surveillance records have identified *A. maculatum* in 62.3% (48/77) counties in Oklahoma (Table 3.5). Active surveillance in summer 2014 confirmed *A. maculatum* in 5 counties and added two previously unreported counties to the current distribution (Figure 3.4). Overall, *A. maculatum* has been reported (n=40) or established (n=10) in 64.9% (50/77) counties in Oklahoma (Table 3.4).

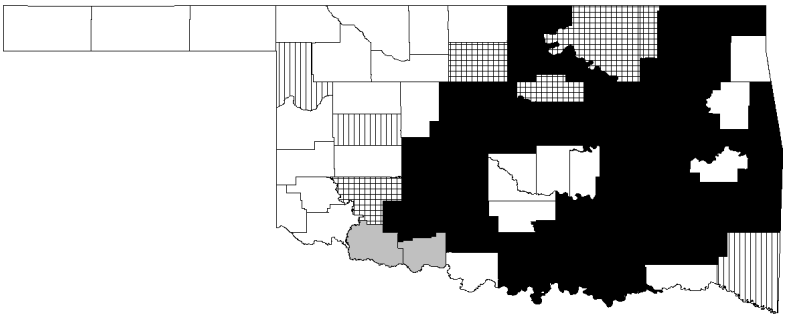

Figure 3.4. Distribution of *A. maculatum* at the county level across the State of Oklahoma. Counties in black = Peer-reviewed, Grey = Active collection, 2014, Crossfill = both methods.

DISCUSSION

Establishing baseline distribution maps and updating historical maps are critical to understanding how tick populations are spread over diverse eco-zones as well as evaluating if there is any change in the potential risk for specific tick-borne diseases in a specific geographic area. Given the dramatic increase of tick-borne diseases in human and animal populations in Oklahoma over the last 15 years (Openshaw et al. 2010; Dahlgren et al. 2011; Starkey et al. 2013; Little et al. 2014; Carmichael et al. 2014), it is critical to understand where the tick species are distributed that are responsible for

transmitting specific pathogens. By using active surveillance, the current study added an additional 10 counties to the list compiled from published studies for distribution of the American dog tick, *D. variabilis*. *Ixodes scapularis* collected from controlled deer hunts added 4 additional counties to the distribution for *I. scapularis* and indicated that the species has possibly moved westward in the state than previously thought (Dennis et al. 1998). Two new counties were added to the historical distribution of *A. maculatum* and three counties were added to a baseline distribution map for *D. albipictus*. Active surveillance over a 6 month period demonstrated that these four tick species of medical and veterinary importance are more widespread throughout the state than previously thought.

This study established a baseline distribution map for the presence of *D. variabilis* in Oklahoma. An important vector for the transmission of several pathogens that impact human and animal health, *D. variabilis* is a known vector for agents that cause diseases including Rocky Mountain spotted fever (*Rickettsia rickettsii*) (Burgdorfer 1969), afebrile spotted fever group rickettsiosis (SFGR) (*R. montanensis*) (McQuiston et al. 2012), tularemia (*Franciscella tularensis*) (Mani et al. 2012), bovine anaplasmosis (*Anaplasma marginale*) (Kocan et al. 2010), and equine piroplasmosis (*Theileria equi*) (Scoles et al. 2011). Over the past 10-15 years, Oklahoma has become one of the top two states for incidence of SFGR (Openshaw et al. 2010) in addition to ranking 6th for incidence of tularemia (CDC 2013). Anaplasmosis also continues to be significant concern for livestock in the state (Wright et al. 1985; J Talley, Extension Livestock Entomologist, personal communication). Establishing the presence of *D. variabilis* throughout most of the state, including the arid western counties as well as the moist, humid eastern counties,

is a first step to identifying the ecological factors involved with maintaining this important tick vector species in the region.

This study also established a baseline distribution map for *D. albipictus*, a one-host tick found on horses (Duell et al. 2013), cattle (Polito et al. 2013), elk (Patrick & Hair 1975), white-tailed deer (Hunt & Gilbert 1981), and feral swine (Sanders et al. 2013) in the Oklahoma/Texas region. A known vector for *A. marginale*, the causative agent of bovine anaplasmosis (Ewing et al. 1997; Kocan et al. 2010), very little is known concerning the ecology and habits of this tick in the Great Plains regions, especially in regards to interactions with cattle (Drummond 1967).

Active surveillance through control deer hunts in western counties extended the known distribution of *I. scapularis* further west in the state than previously reported (Dennis et al. 1998). One of the most important tick vectors of disease agents in the United States, this species is well known to transmit the agents of Lyme disease (*Borrelia burgdorferi*), human granulocytic anaplasmosis (*A. phagocytophilum*), human babesiosis (*Babesia microti*), and *Borrelia miyamotoi* in the north-central and north eastern United States and California (Hamer et al. 2012; Salkeld et al. 2014). The identification of this important species on deer collected in a wildlife management area in the arid, dry western portion of Oklahoma should not be taken as evidence of these disease agents in the region, but does provide an interesting ecological question for future research.

The authors recognize that a significant portion of this study was taken from active surveillance methods used to track the presence of *A. americanum* throughout the state (Barrett et al. 2015). As such, the flagging and CO_2 methods utilized were not ideal

for collection of the four species reported in this paper (Semtner & Hair 1975; Ginsberg & Ewing 1989). However, given that limitation, it was notable that all species, other than *D. albipictus,* were collected using these sampling techniques. This indicates that when used as part of an active surveillance strategy, these two sampling methods are able to draw enough adult ticks from the surrounding area to establish distribution in a given location (Semtner & Hair 1975). Another limitation encountered in the successful surveillance of *I. scapularis* and *D. albipictus* involved the reduction in ODWC-sponsored deer checking stations during deer hunting season. Oklahoma, like many other states, has converted to an online check process which has significantly changed our ability to readily collect these two important tick species within the state.

In conclusion, an updated distribution of 4 important tick species in Oklahoma was determined within one six month period using active surveillance collection methods and passive monitoring. Baseline distribution maps were developed for *D. variabilis* and *D. albipictus* for the State of Oklahoma and additional counties were added for *I. scapularis* and *A. maculatum*. It is anticipated that these results will assist efforts to better understand the epidemiology of the different diseases associated with these tick species within the Great Plains region (Bartholomew et al. 1995; Cortinas & Spomer 2014). Accurate distribution maps for each species studied will be increasingly important as more tick-borne pathogens are identified and climate changes occur across the central plains of the US.

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REFERENCES CITED

Allen K. E., E. M. Johnson, S. E. Little. 2011. Hepatozoon spp infections in the United States. Vet Clin North Am Small Anim Pract. **41**: 1221-1238

Barker, R. W. 2004. Occurrence of the Gulf Coast Tick (Acari: Ixodidae) on the Wild and Domestic Mammals in North-Central Oklahoma. J. Med. Ento. **41**: 170-178

Barrett, A., S. E. Little, E. Shaw. 2014. "*Rickettsia amblyommii*" and *R. montanensis* Infection in Dogs Following Natural Exposure to Ticks. **14**: 20-25

Barrett, A W., B. H. Noden, J. M. Gruntmeir, T. Holland, J. R. Mitcham, J. E. Martin, E. M. Johnson, S. E. Little. 2015. County Scale Distribution of *Amblyomma americanum* (Ixodida: Ixodidae) in Oklahoma: Addressing Local Deficits in Tick Maps Based on Passive Reporting. J. Med. Entomol. **52**: 269-273

Bartholomew, D. M., W. A. Rowley, M. G. Novak, K. B. Platt. 1995. *Ixodes scapularis* and other ticks (Acari, Ixodidae) associated with Lyme disease in Iowa. Journal of Vector Ecology. **20**: 1-6

Bayles, B. R., B. F. Allan. 2013. Social-ecological factors determine spatial variation in human incidence of tick-borne ehrlichiosis. Epidemiology & Infection. **142**: 1911-1924

Burgdorfer, W. 1969. Ecology of tick vectors of American spotted fever. Bull World Health Organ. **40**: 375-381

Carmichael, R. C., J. R. Duell, T. C. Holbrook, B. H. Herrin, C. M. Leutenegger, T. P. O'Connor, S. E. Little. 2014. Antibodies reactive to Ehrlichia spp. are common in Oklahoma horses. Vector Borne Zoonotic Dis. **14**: 552-556

Centers for Disease Control and Prevention (CDC). 2013. Tularemia - United States, 2001-2010. MMWR Morb Mortal Wkly Rep. **62**: 963-966

Centers for Disease Control and Prevention (CDC). 2015. Tickborne diseases of the U.S. Accessed on 6/8/2015 at http://www.cdc.gov/ticks/diseases/

Clymer, B. C., D. E. Howell, J. A. Hair. 1970. Animal Hosts of Economically Important Ticks (Acarina) in East-Central Oklahoma. Annals of the Entomological Society of America. **63**: 612-613

Cortinas, R, S. M. Spomer. 2014. Occurrence and county-level distribution of ticks (Acari: Ixodoidea) in Nebraska using passive surveillance. J Med Entomol. **51**: 352-359

Dahlgren, F. S., E. J. Mandel, J. W. Krebs, R. F. Massung, J. H. McQuiston. 2011. Increasing Incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000-2007. Am. J. Trop. Med. Hyg. **85**: 124-131

Demma, L. J., R. C. Holman, C. A. Mikosz, A. T. Curns, D. L. Swerdlow, E. L. Paisano, J. E. Cheek. 2006. Rocky Mountain Spotted fever Hospitalizations among American Indians. Am. J. Trop. Med. Hyg. **75**: 537-541

Dennis, D. T., T. S. Nekomoto, J. C. Victor, W. S. Paul, J. Piesman. 1998. Reported Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the United States. J. Med. Entomol. **35**: 629-638

Dergousoff, S. J., T. D. Galloway, L. R. Lindsay, P. S. Curry, N. B. Chilton. 2013. Range Expansion of *Dermacentor variabilis* and *Dermacentor andersoni* (Acari: Ixodidae) Near Their Northern Distributional Limits. Journal of Medical Entomology. **50**: 510-520

Diuk-Wasser, M. A., A. G. Gatewood, M. R. Cortinas, S. Yaremych-Hamer, J. Tsao, U. Kitron, G. Hickling, J. S. Browstein, E. Walker, J. Piesman, D. Fish. 2006. Spatiotemporal Patterns of Host-Seeking *Ixodes scapularis* Nymphs (Acari: Ixodidae) in the United States. J. Med. Entomol. **43**: 166-176

Drummond, R. O. 1967. Seasonal Activity of Ticks (Acarina: Metastigmata) on Cattle in Southwestern Texas. Annals of the Entomological Society of America. **60**: 439-447

Duell, J. F., R. Carmichael, B. H. Herrin, T. C. Holbrook, J. Talley, S. E. Little. 2013. Prevalence and Species of Ticks on Horses in Central Oklahoma. J. Med. Entomol. **50**: 1330-1333

Eddy, G. W. 1940. The Ticks of Oklahoma. Master's thesis. Oklahoma Agricultural and Mechanical College

Ellis Jr., L. L. 1955. A Survey of the Ectoparasites of Certain Mammals in Oklahoma. Ecology. **36**: 12-18

Ewing, S. A., R. J. Panciera, K. M. Kocan, N. L. Ge, R. J. Welsh, R. W. Olson, R. W. Barker, L. E. Rice. 1997. A winter outbreak of anaplasmosis in a nonendemic area of Oklahoma: a possible role for *Dermacentor albipictus*. J. Vet Diagn Invest. **9**: 206-208

Folkema, A. M., R. C. Holman, F. S. Dahlgren, J. E. Cheek, J. H. McQuiston. 2012. Epidemiology of Ehrlichiosis and Anaplasmosis among American Indians in the United States, 2000-2007. Am. J. Trop. Med. Hyg. **87**: 529-537

Gage, Kenneth L., C. E. Hopla, T. G. Schwan. 1992. Cotton Rats and Other Small Mammals as Hosts for Immature *Dermacentor variabilis* (Acari: Ixodidae) in Central Oklahoma. J. Med. Entomol. **29**: 832-842

Ginsberg, H. S., C. P. Ewing. 1989. Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari:Ixodidae). Exp Appl Acarol. **7**: 313-322

Hairgrove, T., M. E. Schroeder, C. M. Budke, S. Rodgers, C. Chung, M. W. Ueti, M. A. Bounpheng. 2015. Molecular and serological in-herd prevalence of *Anaplasma marginale* infection in Texas cattle. Preventive Veterinary Medicine. **119**: 1-9

Hamer, S. A., J. I. Tsao, E. D. Walker, G. J. Hickling. 2010. Invasion of the Lyme Disease Vector *Ixodes scapularis*: Implications for *Borrelia burgdorferi* Endemicity. Eco Health. **7**: 47-63

Hamer, S. A., G. J. Hickling, J. L. Sidge, E. D. Walker, J. I. Tsao. 2012. Synchronous phenology of juvenile *Ixodes scapularis*, vertebrate host relationships, and associated patterns of *Borrelia burgdorferi* ribotypes in the midwestern United States. Ticks Tick Borne Dis. **3**: 65-74

Holman, R. C., J. H. McQuiston, D. L. Haberling, J. E. Cheek. 2009. Increasing Incidence of Rocky Mountain spotted fever among the American Indian Population in the United States. Am. J. Trop. Med. Hyg. **80**: 601-605

Hunt, L. M., B. N. Gilbert. 1981. Ticks Found on White-tailed Deer from Different Ecological Areas of Texas. Southwestern Entomologist. **64**: 341-345

Jobe, D. A., J. A. Nelson, M. D. Adam, S. A. Martin Jr. 2007. Lyme disease in Urban Areas, Chicago. Emerging Infectious Diseases. **13**: 1799-1800

Keirans, J. E., T. R. Litwak. 1989. Pictorial Key to the Adults of Hard Ticks, Family Ixodidae (Ixodida: Ixodoidea), East of the Mississippi River. J. Med. Entomol. **26**: 435-448

Keirans, J. E., L. A. Durden. 1998. Illustrated Key to Nymphs of the Tick Genus *Amblyomma* (Acari: Ixodidae) Found in the United States. J. Med. Entomol. **35**: 489-495

Kocan, K. M., J. de la Fuente, E. F. Blouin, J. F. Coetzee, S. A. Ewing. 2010. The Natural History of *Anaplasma marginale*. Veterinary Parasitology. **167**: 95-107

Koch, H. G., J. E. Dunn. 1980. Ticks collected from small and medium-sized wildlife hosts in LeFlore County, Oklahoma. The Southwestern Entomologist. **5**: 214-221

Koch, H. G. 1982. Seasonal Incidence and Attachment Sites of Ticks (Acari: Ixodidae) on Domestic Dogs in Southeastern Oklahoma and Northwestern Arkansas, USA. J. Med. Entomol. **19**: 293-298

Lee, X., K. Hardy, D. H. Johnson, S. M. Paskewitz. 2013. Hunter-killed Deer Surveillance to Assess Changes in the Prevalence and Distribution of *Ixodes scapularis* (Acari: Ixodidae) in Wisconsin. J. Med. Entomol. **50**: 632-639

Little, S. E., M. J. Beall, D. D. Bowman, R. Chandrashekar, J. Stamaris. 2014. Canine infection with *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma spp*., and *Ehrlichia spp*. in the United States, 2010-2012. Parasit Vectors. **7**:257

Mani, R. J., M. V. Reinchard, R. J. Morton, K. M. Kocan, K. D. Clinkenbeard. 2012. Biology of *Franeisella tularensis* Subspecies *holarctica* Live Vaccine Strain in the Tick Vector *Dermacentor variabilis*. PLoS ONE **7**: e35441

McQuiston, J. H., G. Zemtsova, J. Perniciaro, M. Hutson, J. Singleton, W. L. Nicholson, M. L. Levin. 2012. Afebrile Spotted Fever Group Rickettsia Infection after a Bite from a *Dermacentor variabilis* Tick Infected with *Rickettsia montanensis*. Vector-Borne and Zoonotic Diseases **12**: 1-3

Openshaw, J. J., D. L. Swerdlow, J. W. Krebs, R. C. Holman, E. Mandel, A. Harvey, D. Haberling, R. F. Massung, J. H. McQuiston. 2010. Rocky Mountain Spotted Fever in the United States, 2000-2007: Interpreting Contemporary Increases in Incidence. Am. J. Trop. Med. Hyg. **83**: 174-182

Parrish, D. W. 1970. Fleas, Ticks, and Lice Infesting Dogs in North-Central Oklahoma. The Oklahoma Veterinarian. **22**: 15-18

Patrick, C. D., J. A. Hair. 1975. Ecological Observations on *Dermacentor Albipictus* (Packard) in Eastern Oklahoma. J. Med. Entomol. **12**: 393-394

Physiology of Ticks. Current Themes in Tropical Science Vol. 1. Ed. F. D. Obenchain and Rachel Galun. Pergamon Press, Oxford, 1983.Chapter 2

Polito, V. J., K. A. Baum, M. E. Payton, S. E. Little, S. D. Fuhlendorf, M. V. Reichard. 2013. Tick Abundance and Levels of Infestation on Cattle in Response to Patch Burning. Rangeland Ecol Manage. **66**: 545-552

Rand, P. W., E. H. Lacombe, R. Dearborn, B. Cahill, S. Elias, C. B. Lubelczyk, G. A. Beckett, R. P. Smith Jr. 2007. Passive Surveillance in Maine, an Area Emergent for Tick-Borne Diseases. J. Med. Entomol. **44**: 1118-1129

Randolph, S. E. 2008. Tick-borne disease systems. Rev. sci. tech. Off. Int. Epiz. **27**(2): 00-00

Rydzewski, J., N. Mateus-Pinilla, R. E. Warner, J. A. Nelson, T. C. Velat. 2012. *Ixodes scapularis* (Acari: Ixodidae) Distribution Surveys in the Chicago Metropolitan Region. J. Med. Entomol. **49**: 955-959

Sanders, D. M., A. L. Schuster, P. W. McCadle, O. F. Strey, T. L. Blankenship, P. D Teel. 2013. Ixodid Ticks Associated with Feral Swine in Texas. Journal of Vector Ecology. **38**: 361-373

Salkeld, D. J., S. Cinkovich, N. C. Nieto. 2014. Tick-borne pathogens in northwestern California, USA. Emerg Infect Dis **20**: 493-494

Scoles, G. A., H. J. Hutcheson, J. L. Schlater, S. G. Hennager, A. M. Pelzel, D. P. Knowles. 2011. Equine Piroplasmosis Associated with *Amblyomma cajennense* Ticks, Texas, USA. Emerging Infectious Diseases. **17**: 1903-1905

Semtner, P. J., J. A. Hair. 1975. Evaluation of CO2-Baited Traps for Survey of *Amblyomma maculatum* Koch and *Dermacentor variabilis* Say (Acarina: Ixodidae). J. Med. Entomol. **12**: 137-138

Smith, J. S. 1977. A Survey of Ticks Infesting White-tailed Deer in 12 Southeastern States. Master's thesis. University of Georgia

Springer, Y. P., L. Eisen, L. Beati, A. M. James, R. J. Eisen. 2014. Spatial Distribution of Counties in the Continental United States with Records of Occurrence of *Amblyomma americanum* (Ixodida: Ixodidae). J. Med. Entomol. **52**: 342-351

Starkey, L. A., M. D. West, A. W. Barrett, J. M. Saucier, T. P. O'Connor, K. L. Paras, M. H. Reiskind, M. V. Reichard, S. E. Little. 2013. Prevalence of antibodies to spotted fever group *Rickettsia spp*. and *Ehrlichia spp*. in coyotes (*Canis latrans*) in Oklahoma and Texas, USA. J Wildl Dis. **49**: 670-673

Stromdahl, E. Y., J. Jiang, M. Vince, A. L. Richards. 2011. Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group Rickettsiae in the United States. Vector Borne Zoonotic Dis. **11**: 969-977

Vaughn, M. F., J. Delisle, J. Johnson, G. Daves, C. Williams, J. Reber, N. L. Mendell, D. H. Bouyer, W. L. Nicholson, A. C. Moncayo, S. R. Meshnick. 2014. Seroepidemiologic study of human infections with spotted fever group Rickettsiae in North Carolina. J Clin Microbiol. **52**: 3960-3966

Wang, P., M. N. Glowacki, A. E. Hoet, G. R. Needham, K. A. Smith, R. E. Gary, X. Li. 2014. Emergence of *Ixodes scapularis* and *Borrelia burgdorferi*, the Lyme disease vector and agent, in Ohio. Frontiers in Cellular and Infection Microbiology. **4**: 1-9

Wright, J. C., K. M. Kocan, S. A. Ewing, B. H. Espe. 1985. Oklahoma Anaplasmosis Survey. OVMA. **37**: 130-132

Appendix to Chapter III:

Table 3.1. List of publications used to determine reported presence of *D. variabilis, D. albipictus, I. scapularis, and A. maculatum* from peer-reviewed sources.

Dermacentor variabilis	Unknown	Eddy, 1940
	Various mammals examined	Ellis, 1955
	Various animals examined	Clymer et al. 1969
	Stray Dogs examined	Parrish, 1970
		Koch & Dunn 1980
	Dogs examined	Koch, 1982
	Small mammals examined	Gage et al. 1992
	Dogs examined	Barrett et al. 2014
	CO_2 Traps	Semtner & Hair 1975
Dermacentor albipictus	Unknown	Eddy, 1940
	Elk examined	Patrick & Hair 1975
	Collected from harvested white-tailed deer	Smith, 1977
	Cattle examined	Ewing et al. 1997
	Dragging, cattle examined	Polito et al. 2013
Ixodes scapularis	Unknown	Eddy, 1940
	Collected from harvested white-tailed deer	Smith, 1977
	Flagging, dragging, deer surveys, small-medium sized mammal surveys, CO_2 baiting, and tick submissions	Dennis et al. 1998
Amblyomma maculatum	Collected from harvested white-tailed deer	Smith, 1977
	Collected from various hosts	Barker et al. 2004

Table 3.2. Counties in Oklahoma where *D. variabilis* has been either Reported [R] or Established E.

Counties	Peer-reviewed Literature	VectorMap	K.C. Emerson Entomology Museum	USDA-CEIR	Active collection in 2014-2015	Current Status of County
		Number of reports	Number of ticks		Number of ticks	
Adair	[R]	0	1	NR	ND	Reported
Alfalfa	NR	0	NR	NR	ND	
Atoka	NR	0	2	[R]	ND	Reported
Beaver	NR	0	NR	NR	ND	
Beckham	NR	0	NR	NR	1	Reported
Blaine	[R]	0	>6	[R]	1	Establ.
Bryan	NR	0	1	NR	ND	Reported
Caddo	NR	0	>6	[R]	6	Establ.
Canadian	NR	0	NR	NR	ND	
Carter	[R]	0	2	NR	ND	Reported
Cherokee	[R]	0	NR	NR	ND	Reported
Choctaw	[R]	0	NR	E	ND	Establ.
Cimarron	NR	0	NR	NR	ND	
Cleveland	[R]	0	2	NR	14	Establ.
Coal	NR	0	1	NR	1	Reported
Comanche	[R]	10	1	NR	ND	Reported
Cotton	NR	0	NR	NR	3	Reported
Craig	[R]	0	1	NR	ND	Reported
Creek	[R]	0	NR	NR	ND	Reported
Custer	NR	0	NR	NR	3	Reported
Delaware	[R]	0	NR	NR	5	Reported
Dewey	NR	0	NR	[R]	2	Reported
Ellis	NR	0	1	NR	1	Reported
Garfield	[R]	2	NR	NR	6	Establ.
Garvin	NR	0	1	NR	2	Reported
Grady	NR	0	NR	NR	6	Establ.
Grant	NR	0	NR	NR	2	Reported
Greer	NR	0	NR	NR	ND	
Harmon	NR	0	NR	NR	3	Reported
Harper	NR	0	>6	[R]	ND	Establ.
Haskell	[R]	0	NR	NR	ND	Reported
Hughes	NR	0	NR	NR	1	Reported
Jackson	NR	4	3	[R]	ND	Reported
Jefferson	NR	0	>6	NR	2	Establ.
Johnston	[R]	0	NR	NR	ND	Reported
Kay	[R]	0	NR	NR	ND	Reported
Kingfisher	NR	0	1	NR	4	Reported
Kiowa	[R]	0	NR	NR	1	Reported
Latimer	[R]	0	NR	NR	ND	Reported
Le Flore	[R]	1	NR	NR	1	Reported
Lincoln	[R]	0	>6	[R]	ND	Establ.
Logan	[R]	0	>6	[R]	ND	Establ.
Love	NR	0	NR	NR	2	Reported

Counties	Peer-reviewed Literature	VectorMap	K.C. Emerson Entomology Museum	USDA-CEIR	Active collection in 2014-2015	Current Status of County
		Number of reports	Number of ticks		Number of ticks	
Major	NR	0	>6	NR	1	Establ.
Marshall	[R]	0	>6	[R]	ND	Establ.
Mayes	NR	0	>6	E	ND	Establ.
McClain	[R]	0	NR	NR	3	Reported
McCurtain	[R]	1	NR	E	ND	Establ.
McIntosh	[R]	0	NR	NR	ND	Reported
Murray	[R]	0	NR	NR	3	Reported
Muskogee	[R]	1	1	NR	ND	Reported
Noble	[R]	0	>6	[R]	ND	Establ.
Nowata	[R]	0	NR	NR	ND	Reported
Okfuskee	[R]	0	NR	NR	ND	Reported
Oklahoma	[R]	0	4	[R]	ND	Reported
Okmulgee	NR	0	2	NR	ND	Reported
Osage	[R]	0	NR	NR	8	Establ.
Ottawa	[R]	0	1	NR	ND	Reported
Pawnee	[R]	0	1	E	8	Establ.
Payne	[R]	3	>6	E	ND	Establ.
Pittsburg	[R]	3	NR	NR	ND	Reported
Pontotoc	NR	0	NR	NR	ND	
Pottawatomie	NR	0	NR	NR	5	Reported
Pushmataha	[R]	0	NR	E	ND	Establ.
Roger Mills	NR	0	NR	NR	1	Reported
Rogers	[R]	0	1	NR	ND	Reported
Seminole	NR	0	NR	NR	ND	
Sequoyah	[R]	0	NR	[R]	ND	Reported
Stephens	NR	0	1	NR	2	Reported
Texas	NR	0	1	NR	ND	Reported
Tillman	NR	0	NR	NR	ND	
Tulsa	NR	0	1	[R]	ND	Reported
Wagoner	NR	0	NR	NR	ND	
Washington	[R]	0	NR	NR	1	Reported
Washita	NR	0	1	NR	1	Reported
Woods	NR	0	>6	NR	ND	Establ.
Woodward	[R]	0	NR	NR	1	Reported

R-Reported
NR-Not reported
ND- Not determined

Table 3.3. Counties where *D. albipictus* has been either Reported [R] or Established E.

Counties	Peer-reviewed Literature*	K.C. Emerson Entomology Museum	USDA CEIR	Active collection in 2014-2015	Current Status of County
		Number of ticks		Number of ticks	
Adair	NR	1	[R]	ND	Reported
Alfalfa	NR	NR	NR	ND	
Atoka	NR	NR	[R]	ND	Reported
Beaver	NR	NR	NR	ND	
Beckham	NR	NR	NR	ND	
Blaine	NR	NR	NR	14	Establ.
Bryan	[R]	NR	NR	ND	Reported
Caddo	NR	NR	NR	ND	
Canadian	NR	NR	[R]	ND	Reported
Carter	NR	2	NR	ND	Reported
Cherokee	[R]	NR	E	ND	Establ.
Choctaw	NR	NR	[R]	ND	Reported
Cimarron	NR	NR	NR	ND	
Cleveland	NR	NR	NR	ND	
Coal	NR	NR	NR	ND	
Comanche	[R]	>6	E	ND	Establ.
Cotton	NR	NR	NR	ND	
Craig	NR	NR	NR	ND	
Creek	NR	1	NR	ND	Reported
Custer	NR	NR	NR	ND	
Delaware	[R]	NR	NR	ND	Reported
Dewey	NR	NR	[R]	14	Establ.
Ellis	NR	NR	NR	ND	
Garfield	NR	5	NR	ND	Reported
Garvin	NR	NR	[R]	ND	Reported
Grady	NR	NR	NR	ND	
Grant	NR	NR	NR	ND	
Greer	NR	NR	NR	ND	
Harmon	NR	NR	NR	ND	
Harper	[R]	>6	NR	ND	Establ.
Haskell	NR	NR	[R]	ND	Reported
Hughes	NR	NR	NR	ND	
Jackson	NR	NR	NR	ND	
Jefferson	NR	NR	NR	ND	
Johnston	NR	>6	[R]	ND	Establ.
Kay	NR	NR	NR	ND	
Kingfisher	NR	NR	NR	ND	
Kiowa	NR	NR	NR	ND	
Latimer	[R]	NR	[R]	ND	Reported
Le Flore	[R]	NR	E	ND	Establ.
Lincoln	NR	NR	NR	ND	
Logan	NR	NR	NR	ND	
Love	NR	NR	NR	ND	

Counties	Peer-reviewed Literature*	K.C. Emerson Entomology Museum	USDA CEIR	Active collection in 2014-2015	Current Status of County
		Number of ticks		Number of ticks	
Major	NR	NR	NR	14	Establ.
Marshall	NR	NR	[R]	ND	Reported
Mayes	NR	NR	E	ND	Establ.
McClain	NR	NR	NR	ND	
McCurtain	[R]	>6	[R]	ND	Establ.
McIntosh	NR	NR	NR	ND	
Murray	[R]	NR	[R]	ND	Reported
Muskogee	NR	NR	[R]	ND	Reported
Noble	NR	1	[R]	ND	Reported
Nowata	NR	NR	NR	ND	
Okfuskee	NR	NR	NR	ND	
Oklahoma	NR	NR	NR	ND	
Okmulgee	NR	>6	NR	ND	Establ.
Osage	NR	NR	E	ND	Establ.
Ottawa	NR	NR	NR	ND	
Pawnee	[R]	NR	NR	ND	Reported
Payne	NR	4	E	ND	Establ.
Pittsburg	[R]	NR	[R]	ND	Reported
Pontotoc	NR	2	NR	ND	Reported
Pottawatomie	NR	NR	NR	ND	
Pushmataha	[R]	NR	E	ND	Establ.
Roger Mills	NR	NR	NR	ND	
Rogers	NR	NR	NR	ND	
Seminole	NR	NR	NR	ND	
Sequoyah	NR	>6	[R]	ND	Establ.
Stephens	NR	NR	[R]	ND	Reported
Texas	NR	NR	NR	ND	
Tillman	NR	NR	NR	ND	
Tulsa	NR	NR	NR	ND	
Wagoner	NR	NR	NR	ND	
Washington	NR	NR	NR	ND	
Washita	NR	NR	NR	ND	
Woods	NR	NR	NR	ND	
Woodward	NR	NR	NR	ND	

R-Reported
NR-Not reported
ND- Not determined
E-Established prev.

Table 3.4. Counties where *I. scapularis* has been either Reported [R] or Established E.

Counties	Peer-reviewed Literature	K.C. Emerson Entomology Museum	USDA-CEIR	Active collection in 2014-2015	Current Status of County
		Number of ticks		Number of ticks	
Adair	[R]	NR	NR	ND	Reported
Alfalfa	NR	NR	NR	ND	
Atoka	[R]	NR	E	ND	Establ.
Beaver	NR	NR	NR	ND	
Beckham	NR	NR	NR	ND	
Blaine	NR	NR	NR	9	Establ.
Bryan	[R]	NR	NR	ND	Reported
Caddo	[R]	NR	NR	ND	Reported
Canadian	NR	NR	NR	ND	
Carter	[R]	>6	NR	ND	Establ.
Cherokee	E	>6	[R]	ND	Establ.
Choctaw	NR	NR	NR	ND	
Cimarron	NR	NR	NR	ND	
Cleveland	NR	1	NR	100	Establ.
Coal	[R]	NR	NR	ND	Reported
Comanche	[R]	>6	E	ND	Establ.
Cotton	NR	NR	NR	ND	
Craig	[R]	NR	NR	ND	Reported
Creek	[R]	4	NR	1	Reported
Custer	NR	NR	NR	14	Establ.
Delaware	[R]	NR	NR	1	Reported
Dewey	NR	NR	NR	9	Establ.
Ellis	NR	NR	NR	ND	
Garfield	[R]	3	NR	ND	Reported
Garvin	[R]	NR	NR	ND	Reported
Grady	NR	5	NR	5	Reported
Grant	NR	NR	NR	ND	
Greer	NR	NR	NR	ND	
Harmon	NR	NR	NR	ND	
Harper	NR	NR	NR	ND	
Haskell	[R]	NR	NR	ND	Reported
Hughes	[R]	NR	NR	ND	Reported
Jackson	NR	NR	NR	ND	
Jefferson	NR	NR	NR	ND	
Johnston	[R]	NR	NR	ND	Reported
Kay	NR	NR	NR	ND	
Kingfisher	NR	NR	NR	ND	
Kiowa	NR	NR	NR	ND	
Latimer	[R]	>6	[R]	ND	Establ.
Le Flore	E	NR	NR	ND	Establ.
Lincoln	[R]	1	NR	ND	Reported
Logan	[R]	NR	NR	5	Reported
Love	[R]	NR	NR	ND	Reported

Counties	Peer-reviewed Literature	K.C. Emerson Entomology Museum	USDA-CEIR	Active collection in 2014-2015	Current Status of County
		Number of ticks		Number of ticks	
Major	NR	NR	NR	9	Establ.
Marshall	NR	NR	NR	ND	
Mayes	[R]	6	[R]	ND	Establ.
McClain	E	NR	NR	ND	Establ.
McCurtain	[R]	5	[R]	ND	Reported
McIntosh	[R]	NR	NR	ND	Reported
Murray	[R]	NR	NR	ND	Reported
Muskogee	[R]	NR	[R]	ND	Reported
Noble	NR	4	NR	ND	Reported
Nowata	NR	NR	NR	ND	
Okfuskee	[R]	NR	NR	ND	Reported
Oklahoma	[R]	1	NR	ND	Reported
Okmulgee	[R]	NR	NR	2	Reported
Osage	NR	NR	NR	ND	
Ottawa	[R]	4	NR	ND	Reported
Pawnee	[R]	NR	NR	1	Reported
Payne	[R]	>6	[R]	ND	Establ.
Pittsburg	[R]	6	[R]	ND	Establ.
Pontotoc	[R]	NR	NR	ND	Reported
Pottawatomie	[R]	NR	NR	ND	Reported
Pushmataha	[R]	1	NR	ND	Reported
Roger Mills	NR	NR	NR	ND	
Rogers	[R]	1	NR	ND	Reported
Seminole	[R]	2	NR	ND	Reported
Sequoyah	[R]	6	E	ND	Establ.
Stephens	NR	NR	[R]	ND	Reported
Texas	NR	NR	NR	ND	
Tillman	NR	NR	NR	ND	
Tulsa	[R]	1	[R]	6	Establ.
Wagoner	[R]	1	NR	ND	Reported
Washington	[R]	NR	NR	ND	Reported
Washita	NR	NR	NR	ND	
Woods	NR	NR	NR	ND	
Woodward	NR	NR	NR	ND	

R-Reported
NR-Not reported
ND- Not determined
E-Established

Table 3.5. Counties where *A. maculatum* has been either Reported [R] or Established E.

Counties	Peer-reviewed Literature	K.C. Emerson Entomology Museum	USDA CEIR	Active collection in 2014-2015	Current Status of County
		Number of ticks		Number of ticks	
Adair	[R]	NR	NR	ND	Reported
Alfalfa	NR	NR	NR	ND	
Atoka	[R]	NR	NR	ND	Reported
Beaver	NR	NR	NR	ND	
Beckham	NR	NR	NR	ND	
Blaine	NR	NR	NR	ND	
Bryan	[R]	NR	NR	ND	Reported
Caddo	[R]	2	NR	ND	Reported
Canadian	[R]	2	NR	ND	Reported
Carter	[R]	>6	NR	ND	Establ.
Cherokee	NR	NR	NR	ND	
Choctaw	NR	NR	NR	ND	
Cimarron	NR	NR	NR	ND	
Cleveland	NR	NR	NR	ND	
Coal	[R]	4	NR	ND	Reported
Comanche	[R]	NR	NR	ND	Reported
Cotton	NR	NR	NR	3	Reported
Craig	[R]	2	NR	ND	Reported
Creek	[R]	1	NR	ND	Reported
Custer	NR	1	NR	ND	Reported
Delaware	NR	NR	NR	ND	
Dewey	NR	NR	NR	ND	
Ellis	[R*]	1	NR	ND	Reported
Garfield	[R]	1	NR	1	Reported
Garvin	NR	NR	NR	ND	
Grady	[R]	4	NR	ND	Reported
Grant	NR	NR	NR	ND	
Greer	NR	NR	NR	ND	
Harmon	NR	NR	NR	ND	
Harper	NR	NR	NR	ND	
Haskell	NR	NR	NR	ND	
Hughes	[R]	3	NR	ND	Reported
Jackson	NR	NR	NR	ND	
Jefferson	NR	NR	NR	ND	
Johnston	NR	2	[R]	ND	Reported
Kay	[R]	>6	NR	ND	Establ.
Kingfisher	[R]	1	NR	ND	Reported
Kiowa	NR	1	NR	1	Reported
Latimer	[R]	1	NR	ND	Reported
Le Flore	[R]	NR	NR	ND	Reported
Lincoln	[R]	NR	NR	ND	Reported
Logan	[R]	2	NR	ND	Reported
Love	[R]	1	NR	ND	Reported

Counties	Peer-reviewed Literature	K.C. Emerson Entomology Museum	USDA CEIR	Active collection in 2014-2015	Current Status of County
		Number of ticks		Number of ticks	
Major	NR	NR	NR	ND	
Marshall	[R]	>6	NR	ND	Establ.
Mayes	[R]	>6	[R]	ND	Establ.
McClain	NR	NR	NR	ND	
McCurtain	NR	2	NR	ND	Reported
McIntosh	[R]	2	NR	ND	Reported
Murray	[R]	NR	NR	ND	Reported
Muskogee	[R]	>6	NR	ND	Establ.
Noble	[R]	1	[R]	ND	Reported
Nowata	[R]	NR	[R]	ND	Reported
Okfuskee	[R]	1	NR	ND	Reported
Oklahoma	[R]	4	NR	ND	Reported
Okmulgee	[R]	1	NR	ND	Reported
Osage	[R]	1	NR	16	Establ.
Ottawa	[R]	NR	NR	ND	Reported
Pawnee	[R]	NR	[R]	ND	Reported
Payne	[R]	>6	NR	2	Establ.
Pittsburg	[R]	6	NR	ND	Establ.
Pontotoc	[R]	NR	NR	ND	Reported
Pottawatomie	NR	NR	NR	ND	
Pushmataha	[R]	NR	NR	ND	Reported
Roger Mills	NR	NR	NR	ND	
Rogers	[R]	5	[R]	ND	Reported
Seminole	NR	NR	NR	ND	
Sequoyah	[R]	NR	NR	ND	Reported
Stephens	[R]	6	NR	ND	Establ.
Texas	NR	NR	NR	ND	
Tillman	NR	NR	NR	1	Reported
Tulsa	[R]	4	NR	ND	Reported
Wagoner	[R]	>6	[R]	ND	Establ.
Washington	[R]	1	[R]	1	Reported
Washita	NR	NR	NR	ND	
Woods	NR	NR	NR	ND	
Woodward	NR	NR	NR	ND	

*Personal Com.
R-Reported
NR-Not reported
ND- Not determined
E-Established

CHAPTER IV

TICKS FROM TWO POPULATIONS OF AMERICAN BLACK BEAR (URSUS AMERICANUS) IN EASTERN OKLAHOMA

Abstract

The American black bear (*Ursus americanus*) is an important mega-omnivore that recolonized the Ouachita mountain range and Ozark foothills in the State of Oklahoma in the mid-1980s. The habitat for black bear is also important for several tick species in Oklahoma which are also known vectors for zoonotic diseases. These diseases may impact bear populations as well as other wildlife, livestock, humans and/or companion animals whose habitats or activities overlap with the bear. Currently, there is no information on the tick species parasitizing the black bear in Oklahoma. Between May and August 2014, in conjunction with radio-collaring efforts by the Oklahoma Department of Wildlife and Conservation, 1159 ticks were collected from 62 bear in the Ouachita mountain range and Ozark foothills in Oklahoma. Thirteen adult bear in dens and 22 cubs in dens were monitored during the winter to determine which tick species fed on female bear and their cubs. The primary species of tick in both locations is the Lone Star tick (*Amblyomma americanum*) (69.3%). Other tick species identified were *Dermacentor variabilis* (29.0%), *Ixodes scapularis* (0.345%) and *Amblyomma maculatum* (0.77%). During February and March of 2015, 13 bear and 22 cubs were

monitored at their dens for ectoparasites. No fleas or lice were found and only 3 ticks were collected.

Introduction

The American black bear (*Ursus americanus*) is an important mega-omnivore that can be found in 39 states within the United States (Pelton 2003). Although the species was extirpated from large portions of their historic range because of land settlement, their range has been expanding in recent years (Pelton et al. 1999; Williamson 2002). In the southern US, including the Ozark and Ouachita Mountains, the black bear has been recorded in predominantly oak-hickory and mixed mesophytic forest (Pelton 2003), the exact type of habitat preferred by many of the tick species focused on in this research thesis.

Successful recolonization efforts in Arkansas in the 1950's and 60's lead to the recolonization of bear populations in Oklahoma in the 1980's (Lyda & Hellgren 2007). Oklahoma black bear reside in a variety of habitats including oak-hickory and oak-pine forests (Lyda & Hellgren 2007). In addition to providing ample habitat for thriving bear populations, these habitats are also important for Oklahoma's tick species of medical and veterinary importance. Many of the same tick species found in Oklahoma have been reported on black bear in Louisiana, Georgia, Florida, and Wisconsin (Manville 1978; Yabsley et al. 2009; Leydet & Liang 2013). Bear may be important hosts for these tick species and they may be spreading ticks throughout their home range, bringing ticks into contact with other wildlife, livestock, humans and/or companion animals (Yabsley et al. 2009; Leydet & Liang 2013). Additionally, various tick-borne pathogens including *Rickettsia sp.* and *Ehrlichia chaffeensis* have been associated with ticks collected from

black bear (Yabsley et al. 2009; Leydet & Liang 2013). To date, there is no information published on the tick species that parasitize Oklahoma black bear.

The objective of this study was to investigate which ectoparasites parasitize Oklahoma black bear.

Methods

The sampling of adult bear took place throughout the summer of 2014 and adult bear, their cubs and dens were sampled in late winter 2015.

Summer 2014

Ticks were collected off tranquilized bear in both the Ozark foothills and the Ouachita mountain range (Fig. 4.1) in collaboration with a radio-collaring study by the Oklahoma Department of Wildlife Conservation (ODWC) and the department of Natural Resource Ecology and Management (NREM) at Oklahoma State University.

Figure 4.1. Locations of Oklahoma black bear research, summer 2014 & winter 2015. Northern pin indicates area of Ozark foothills; Southern pin indicates area of Ouachita mountain range (Map source: Google Earth)

Bears were trapped using two methods. The research team in the Ozarks trapped using barrel traps (Fig. 4.2) and the research team in the Ouachita's trapped using foot snares. The success of these trapping methods differed substantially which influenced the collection of ticks significantly.

During May and June 2014, tick sampling efforts alternated between the two sites every two weeks. The month of July and first two weeks of August 2014 was spent entirely with the Ouachita team. The Ouachita team sampled bear with daily field visits, 7 days/week. Since the Ouachita team used foot snares the bear were handled as quickly as possible after capture. Additionally, there were more bear captured in the Ouachita area than the Ozark area with two to three bear handled in one day at times. With the use of snare traps, it was necessary to accompany the team daily in the likelihood of encountering a snared bear.

Figure 4.2. An example of the barrel trap used by the research team in the Ozark area.

Once the bear was tranquilized in either location, the team would collect data which normally provided ample time for sampling for ticks. Main areas searched on the body were the ears, head, neck, and axillary regions and then search the rest of the bear (Fig 4.3, Fig 4.4) (Bloemer et al. 1988). The strategy was to collect a representative sample of ticks at the time of the sampling and levels of infestation were not a focus for this project. When not present to collect ectoparasites, another member of the team at each site would inspect the bear and collect ticks on behalf of the author.

Winter 2015

During denning season (February and March 2015), den visitation was scheduled by both teams so that no den from either location was visited on the same day (Fig 4.5, Fig 4.6). The bear was tranquilized and cubs were removed from den. The cubs were inspected for ectoparasites in all bodily locations mentioned previously. After the cubs were searched, an attempt to reach the female inside the den was made in order to inspect the coat for any ectoparasites. This proved to be challenging as many dens were small and dark. It was difficult to move the female around to be able to check all locations on her body and because the winter fur coat was extremely thick. When the author was not present at a den site, another member of the team would inspect the bear and cubs for parasites.

Due to the nature of the project, ticks were collected when visible since this aspect of the overall project was not the primary goal for trapping the bears. As such, the ticks collected do not represent the total tick populations found on bears. Ticks were placed in vials of 70% EtOH and brought back to the lab to be identified to species and counted.

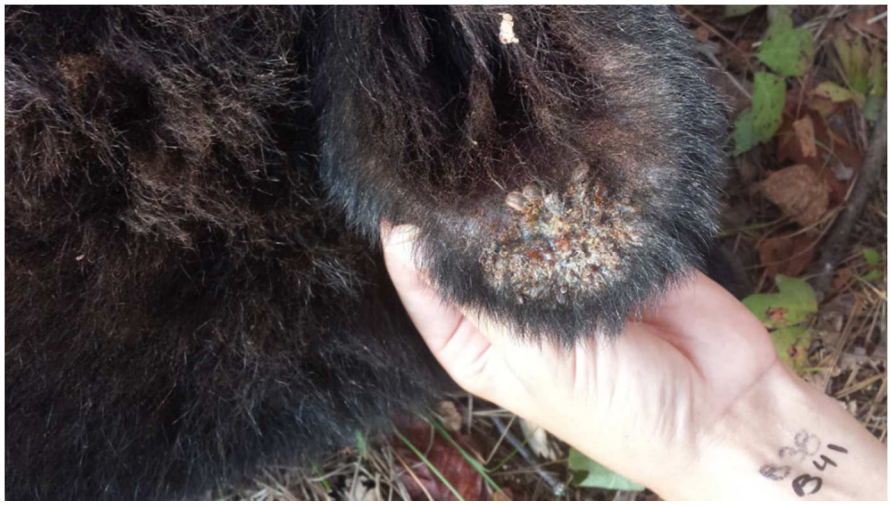

Figure 4.3. An example of ticks found parasitizing an ear of bear #41.


Figure 4.4. The author with bear #32, a 16 year old female, weighing ~185lbs.

Figure 4.5. A.) Replacing a cub with the female bear after a work up at a den located in the Ozark region. B.) Checking a cub for ectoparasites at a den located in the Ouachita region.

Results

A total of 1159 ticks were collected from 62 bears during the months of May-August 2014. *Amblyomma americanum* was the primary species collected. Other species found were *Dermacentor variabilis*, *Ixodes scapularis* and *Amblyomma maculatum* (Table 4.1).

Table 4.1. Tick counts (% collected) for both locations by species and by months with totals.

Tick Species	May (%)	June (%)	July (%)	August (%)	Total Counts (%)
A. americanum	361(88.5%)	288(92.6%)	106(40.7%)	49(27.2%)	804(69.4%)
D. variabilis	40(9.8%)	20(6.4%)	148(56.9%)	129(71.6%)	337(29.1%)
I. scapularis	1(0.24%)	3(0.96%)	0	0	4(0.34%)
A. maculatum	1(0.24%)	0	6(2.3%)	2(1.1%)	9(0.77%)
Other	5(1.22%)	0	0	0	5(0.43%)
TOTAL	**408**	**311**	**260**	**180**	**1159**

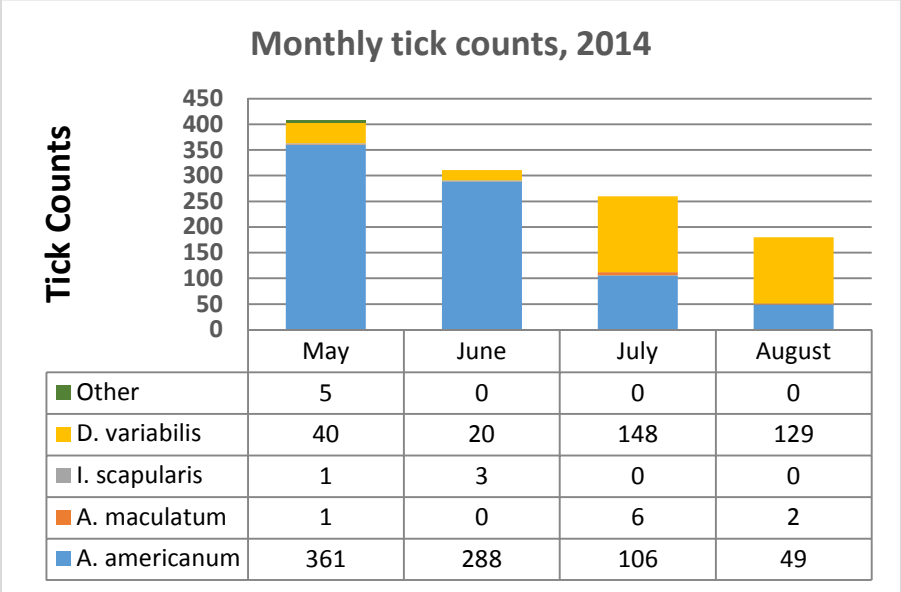

Figure 4.7. The total monthly counts for both locations. "Other" indicates ticks collected minus mouthparts so unable to properly identify.

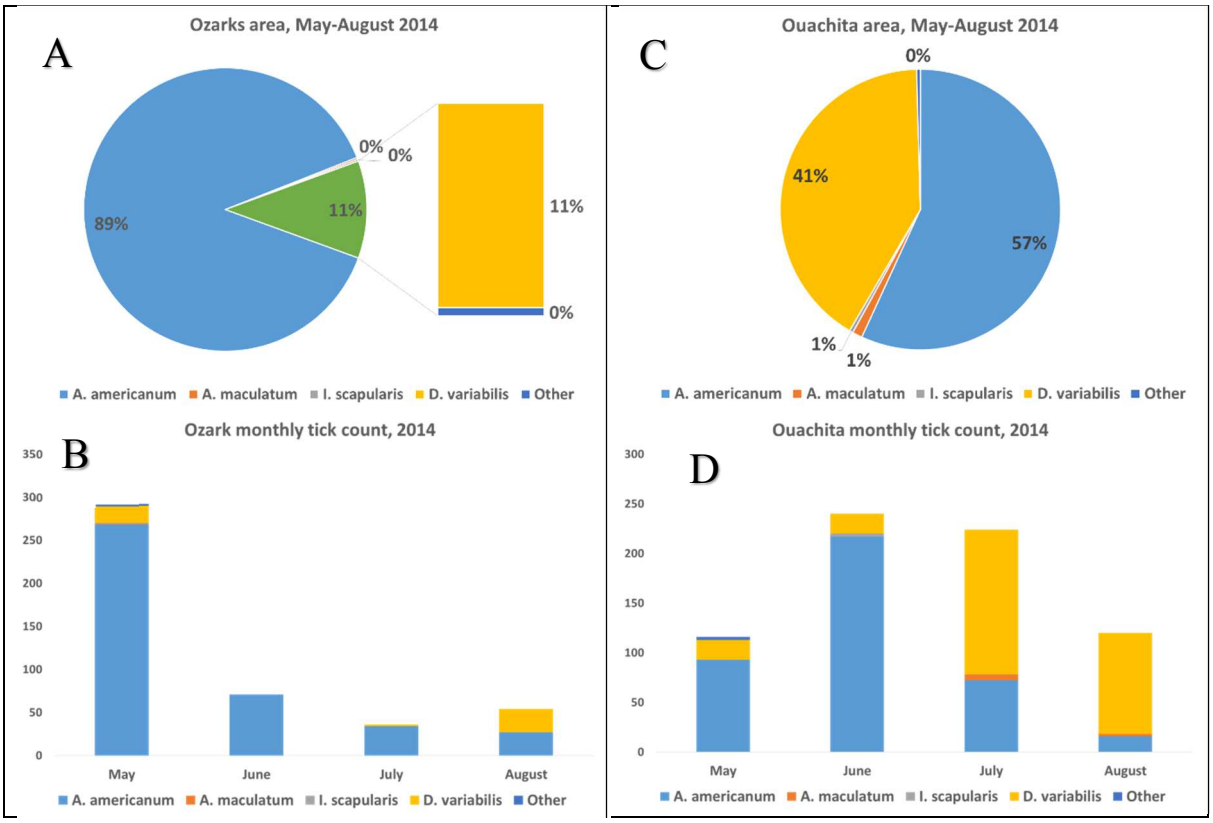

Figure 4.8: A & B) Proportion of tick species collected off bear in Ozark and C & D) Ouachita regions between May and August 2014. A & B) Primary species collected from bear in Ozark was *A. americanum* primarily in May. Ticks were collected from 13 bear. C & D) Primary species collected from bear in Ouachita was *A. americanum* however there was also a high number of *D. variabilis* collected. Ticks were collected from 49 bear.

Combining all ticks collected from all the bear, changes in tick species as the season progressed are apparent (Fig 4.7) The primary species collected from bear were *A. americanum* in the May and June with more *D. variabilis* in July and August with a few *I. scapularis* in May and several *A. maculatum* in July and August. The breakdown of species collected became more obvious when the two areas were separated (Fig 4.8 A-D). In the Ozarks (Fig 4.8 A & B), bear were primarily parasitized by *A. americanum* throughout the season however, in the Ouachitas (Fig 4.8 C & D) the bear were almost equally parasitized by *D. variabilis* in July and August as they were with *A. americanum* in May and June.

During the bear den season, February and March 2015, no ectoparasites were found on any of the 22 cubs while three ticks were found on 3 of the 13 female bear. One male *A. americanum* was collected off an adult denning bear in the Ozark area and two ticks, one male *A. americanum* and one fed *D. variabilis*, were collected from bear in the Ouachita area.

Only one flea, species unidentified, was found on a single bear during the summer trapping. No lice were found on any of the bear.

Discussion

During one summer and one denning season, four species of tick were identified on American black bear populations in Oklahoma. The four species of ticks were expected as they are the four most commonly found tick species parasitizing both humans and animals in the southern US (Leydet & Jiang 2013). During the trapping between the months of May and August 2014, each examined bear was infested with ticks. *Amblyomma americanum* was the most common tick species found on the bears in May and June while *Dermacentor variabilis* was more common in the month of July and August. These findings are consistent with known tick peak activity times (Sonenshine 1972).

While systematic sampling was not achieved during this study, it is interesting to note the differences in total species recovered from bear in both regions. Almost all (89%) ticks recovered from bear in the Ozarks were *A. americanum*, while Lone Star ticks only accounted for 56.9% of the ticks recovered in the Ouachita's. The increased presence of *D. variabilis* in the Ouachita area over the sampling period is notable. This

difference can be observed in species collected by month in both areas summarized in Fig. 4.8. While not conclusive, future studies could be developed to focus on this aspect in more depth.

Some of the expected results, based on previous studies in other areas, were not realized in the current study. It was expected to find lice, specifically *Trichodectes pinguis euarctidos*, on Oklahoma black bear as this louse species was reported on black bear in 10 U.S. states and 2 Canadian provinces (Nims & Durden 2011; Roger & Roger 1976). Additionally, based on a study in Wisconsin (Manville 1978), it was expected to find at least *I. scapularis* on denning bear as bear are denning during peak tick activity times for this species in Oklahoma and several dens were partly exposed or completely exposed to the open (Nims & Durden 2011). Only one study has reported looking for ticks on denning bears (Rogers 1975). Aside from one bear with numerous *D. albipictus* (Rogers 1975), no ectoparasites were reported on denning bear in the literature. This lack of ectoparasites on denning bear may be due to the den location, lack of mobility of bear in which they acquire their ticks, the lack of nidicolous behavior among most hard tick species, or due to the challenge presented by thick winter fur both for ticks to feed and for quick searching strategies deep inside the den.

One of the main limitations to the project was the inability of being at both trapping locations at a given time. For the entire summer, the author had to choose which region to focus on as often a patterned approach was not possible due to major differences in effectiveness of bear trapping methods. When the author was not able to be present at one place, she relied on the goodwill of colleagues who were also very busy with collecting data for their own projects. Often, ticks were collected – but it was not

possible to confirm if it had been done in a systematic way. Also, as is common in field research, it was not possible to evaluate some bear ectoparasites due to different situations such as time constraints and safety of the bear. Because of these two limitations, it was not possible to use the data to make conclusions about tick populations on bear populations in the two geographic locations.

In conclusion, this research reports that Oklahoma black bear are parasitized by at least four of Oklahoma's tick species that are of medical and veterinary importance. The role that the black bears play in pathogen transmission is unknown. However, the wide home ranges of large mammals averaging 14.5-21km^2 (for females, males are expected to have larger home ranges) means these bear have the potential of transporting ticks throughout the Ozark foothills and Ouachita Mountains and these ticks are known vectors for pathogens such as *Ehrlichia spp.* and/or *Rickettsia spp.* (Lyda & Helgren 2007; Leydet & Liang 2013; Mullen & Durden 2009; Springer et al. 2014).

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REFERENCES CITED

Bales, S. L., E. C. Hellgren, D. M. Leslie Jr., J. Hemphill Jr. 2005. Dynamics of a recolonizing population of black bears in the Ouachita Mountains of Oklahoma. The Wildlife Society Bulletin. **33**(4): 1342-1351

Bloemer, S. R., R. H. Zimmerman, K. Fairbanks. 1988. Abundance, Attachment Sites, and Density Estimators of Lone Star Ticks (Acari: Ixodidae) Infesting White-Tailed Deer. J. Med. Entomol. **25**(4): 295-300

Leydet Jr. B. F., F. Liang. 2013. Detection of human bacterial pathogens in ticks collected from Louisiana black bears *(Ursus americanus luteolus).* Ticks and Tick-borne Diseases. **4**: 191-196

Lyda, S. B., E. C. Hellgren. 2007. Diurnal Habitat Selection and Home-range Size of Female Black Bears in the Ouachita Mountains of Oklahoma. Proc. Okla. Acad. Sci. **87**: 55-64

Manville II, A. M. 1978. Ecto-and endoparasites of the black bear in northern Wisconsin. Journal of Wildlife Diseases. **14**(1): 97-101

Mullen, G. R., L. A. Durden. 2009. Medical and Veterinary Entomology. MA: Academic Press. Chapter **26**

Nims, T. N. and L. A. Durden. 2011. Ticks and Lice of the Black Bear, *Ursus americanus* Pallas, in Northern Georgia, USA, Including a New State Record for the Chewing Louse, *Trichodectes euarctidos (Phthiraptera: Trichodectidae).* J. Entomol. Sci. **46**(4): 345-347

Pelton, M. R. 2003. Black Bear. G. A. Feldhammer, J. A. Chapman (Eds.), Wild mammals of North America: biology, management, and conservation. The John Hopkins University Press. Baltimore (2003), pp 547-555

Pelton, M. R., A. B. Coley, T. H. Eason, D. I. Doan Martinez, J. A. Pederson, F. T. van Manen, K. M. Weaver. 1999. American black bear conservation action plan. Pp. 144-156 in C. Servheen, S. Herroro, B. Peyton, compilers. Bears. Status survey and conservation action plan. IUCN/SSC bear and polar bear specialst groups. IUCN, Gland Switzerland and Cambridge, UK

Rogers, L. L. 1975. Parasites of Black Bears of the Lake Superior Region. Journal of Wildlife Disease. **11**: 189-192

Rogers, L. L., S. M. Rogers. 1976. Parasites of Bears: A Review. Their International Conference on Bears. Paper **42**

Sonenshine, D. E. 1972. Ecology of the American Dog Tick, *Dermacentor variabilis,* in a Study Area in Virginia. 1. Studies on Population Dynamics Using Radioecological Methods. Annals of the Entomological Society of America. **65**(5): 1164-1175

Springer, Y. P., L. Eisen, L. Beati, A. M. James, R. J. Eisen. 2014. Spatial Distribution of Counties in the Continental United States with Records of Occurrence of *Amblyomma americanum (Ixodida: Ixodidae).* Journal of Medical Entomology. **51**(2): 342-351

Williamson, D. F. 2002. In the black. Status, management and trade of the American black bear (Ursus americanus) in North America. TRAFFIC North America, World Wildlife Fund, Washington, DC, USA

Yabsley, M. J., T. N. Nims, M. Y. Savage, L. A. Durden. 2009. Ticks and Tick-Borne Pathogens and Putative Symbionts of Black Bears *(Ursus americanus floridanus)* from Georgia and Florida. The Journal of Parasitology. **95**(5): 1125-1128

CHAPTER V

RISK OF EXPOSURE TO TICKS AND TICK-BORNE PATHOGENS IN OKLAHOMA STATE PARKS

Abstract

Oklahoma has one of the highest annual incidence rates for tick-borne diseases in the United States. These include *Ehrlichia chaffeensis* and Rocky Mountain spotted fever in humans and canine ehrlichiosis and hepatozoonosis in companion animals. Oklahoma state parks are well-visited, ranking 15th in the nation for annual visitation. The presence of these tick-borne pathogens and their tick vectors leads to increased risks for park workers and those using the parks for recreational purposes. Given that not all state parks have the same risk levels and are located in varying ecoregions, it was hypothesized that there is a higher risk of encountering ticks and tick-borne pathogens in state parks in the eastern area of the state than in the central or western areas. Between April and August 2015, 1035 ticks were systematically collected from six state parks across Oklahoma by flagging vegetation along state park trails for 20 minutes. During the summer of 2015, 1035 ticks were collected, 71.6% were collected in the eastern area of the state with the remaining 28.4% collected in the state park in central Oklahoma. There was a higher risk for encountering *Amblyomma americanum* (94.2%: 975/1035) and *Ixodes scapularis* ticks (2.2%: 23/1035) during the spring and summer months in the eastern state parks. Only *Dermacentor variabili*s (3.6%; 37/1035) was collected in the western state park.

The collected ticks were tested by PCR for various tick borne pathogens. 9.49% (13/137) of the *A. americanum* pools tested positive for *Ehrlichia chaffeensis* while 67.8% (99/146) of the *A. americanum* and *D. variabilis* pools tested positive for a *Rickettsia spp*. No pools of *I. scapularis* were positive for *Borrelia burgdorferi*. This data provides valuable baseline information into the ecology of ticks and tick-borne disease risk in Oklahoma state parks and provides preliminary data from which to assist the Oklahoma Department of Tourism develop targeted campaigns that substantially reduce disease risk for park visitors.

Introduction

Within any given area of potential transmission of a tick-borne disease, three things must be present: 1) competent hosts which can either be reservoirs for the pathogen or become infected by the pathogen; 2) the pathogen itself in both the hosts and tick vector species; and 3) competent tick species in which the pathogen can develop successfully after being taken up in a blood meal and successfully transmitted to another host. The three components comprise what is often called the 'vector-borne disease triangle' as all three (host, pathogen, and vector) must be found within an environment that is conducive for all the necessary events to occur as well as habitats in which they can thrive and develop. If these components don't interact, there cannot be transmission of tick-borne pathogens within a given area (Sonenshine 1993).

State parks are an excellent resource in which to study vector-borne disease triangles within a given area. State parks are usually areas of habitat that are put aside for wildlife conservation, observation, and interactions between the local wildlife, park

visitors, and park employees (Cilek & Olson 2000). This interaction provides a good opportunity for arthropod vectors of human pathogens to join the interaction leaving humans and pets potentially exposed to ticks and disease (Eisen et al. 2013). Oklahoma is the fourth most ecologically diverse state in the US with a variety of ecoregions found in state parks stretched across the state (okatlas.org 2004). This allows researchers to define to what extent ticks and tick borne pathogens occur in Oklahoma state parks but also to extrapolate outside of state park system to determine which tick species and pathogens are equally a threat across the various ecoregions.

Oklahoma has one of the highest annual incidence rates for tick-borne diseases in the United States (Openshaw et al. 2010). Many of the vectors for such diseases occur during the summer months. Oklahoma state parks are ranked 15th in the nation for annual visitation (Siikamäki 2011; Siderelis et al. 2012). The majority of national park visitations occur in the summer when ticks are active (Eisen et al. 2013). Park visitors engage in outdoor activities (hiking, biking, walking dogs, camping) while in the park which provide opportunities for visitors and their pets to come into contact with ticks (Eisen et al. 2013). There have been only a few studies that have investigated the risk for exposure at the national park level (Eisen et al. 2013) with researchers in some states investigating the risk for exposure at state park systems (Cilek & Olson 2000; Han et al. 2014; Lane 1996; Paskewitz et al. 2001; Rawlings & Teltow 1994).

Little to nothing is published in Oklahoma state parks about the risk of exposure to ticks and tick-borne pathogens for hikers and/or their companion animals. It is likely that not every state park has the same level of tick infestation, however, differences between state parks in different ecoregions have not been systematically studied for their

tick populations or their tick-borne pathogens. Different tick species can vector different tick-borne diseases and are active during different times of the year. In order to determine the risk of exposure to ticks in Oklahoma state parks, it is important to systematically survey for ticks and pathogens by month over the course of a complete summer. Once the extent of potential risks is recorded, it will be possible to develop strategies to help reduce the risk of human and pet exposure to such pests and pathogens (Eisen et. al. 2013). The purpose of this study was to investigate the level of tick infestation and prevalence of tick borne pathogens in 6 different state parks in six different ecoregions across the state of Oklahoma during the summer months of May to August in 2014 and 2015.

Methods & Materials

Preliminary study: During the summer of 2014 (May to August), preliminary data was collected from 8 state parks (Boiling Springs SP, Roman Nose SP, Lake Thunderbird SP, Lake Tenkiller SP, Sequoyah SP, Greenleaf SP, Robbers Cave SP, and Lake Wister SP) to begin correlating tick risk to state park within differing ecoregions of Oklahoma. Hiking and/or biking trails in each park were chosen by consulting park websites on TravelOK.com. Once trails were identified in each state park, monthly visits were attempted to begin accumulating data concerning tick species activities over the course of the summer. In summary, trails were flagged for 30 minutes using a Summer Infant waterproof multi L pad 27" x 36" (Summer Infant). Every 30 seconds, ticks were collected off the flag, as well as off clothing, and placed in vials of 70% EtOH to be identified in the lab at Oklahoma State University.

Based on the preliminary field work achieved in the summer of 2014, six representative state parks were chosen across the state of Oklahoma (Table 5.1). These parks were chosen because of their representative qualities to the overall ecoregion as well as the known risks for encountering various species of ticks (Fig 5.1). This was necessary in order to gain a more complete understanding of potential tick activity and potential exposure during a wider time period and sample ticks using a systematic approach so that comparisons could be made between parks.

Table 5.1. State parks chosen for systematic sampling in summer 2015 based on presence in specific ecoregions in the state (okatlas.org2004).

State Park	County	Ecoregion
Sequoyah SP	Cherokee	Eastern broadleaf forest, Ozark broadleaf forest
Greenleaf SP	Muskogee	Prairie parkland (Temperate)
Lake Wister SP	Le Flore	Southeastern mixed forest, Ouachita mixed forest
Robbers Cave SP	Latimer	Southeastern mixed forest, Prairie parkland
Roman Nose SP	Blaine	Great Plains steppe and shrub
Lake Thunderbird SP	Cleveland	Great Plains steppe and shrub, Prairie parkland

Summer 2015 sampling season: Between April and August 2015, 6 state parks were visited at the beginning of each month. To maximize comparative value across the state, the attempt was made to sample all parks within a 5 to 7 day period with adjustment necessary for changing environmental conditions such as rain and wind. All parks were sampled over the five month period except Lake Wister State Park in which the hiking trail chosen was inaccessible due to the high volume of rain experienced in June. In each state park, the same trail was flagged at each park for 20 minutes using the methods described by Pardanani & Mather (2004). Trails were flagged (using the same flagging material as the preliminary survey) for 30 seconds for 4 sets of 20 (total sampling time = 20 minutes). Flags were checked for ticks every 30 seconds and this flag checking time

was not included in the sampling time. Ticks were collected off the flag, as well as off clothing, and placed in vials of 70% EtOH to be identified and counted in the lab.

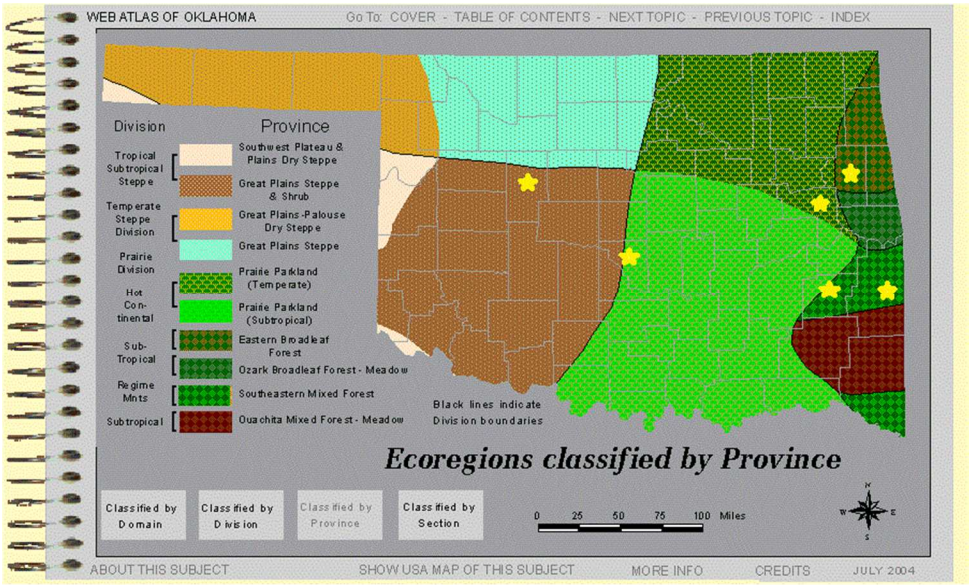

Figure 5.1. State parks sampled during summer 2015 across ecoregions of Oklahoma. Stars used to indicate the locations of the six state parks that were sampled (okatlas.org, 2004).

Analysis of tick exposure in state parks:

Total ticks collected from each state park in each month where divided by the time on the trail (20 minutes) to develop the risk rate of encountering a tick per minute. The flagging method as described by Pardanani & Mather (2004) was used where there was 30sec sampling for 4 sets of 10. This is equal to 20 minutes of sampling time. The flag was checked after every 30 sec sampling for ticks and, if found, was placed in a vial with 70% EtOH.

DNA extraction from tick samples

DNA extraction methods were modified from a previously described extraction method utilized by Salazar (2015). Ticks were washed in two sets of de-ionized water and a final wash in 70% ethanol. After washing, ticks were bisected using razor blades after which the blade was rinsed with 70% ethanol in between individual ticks. One half

of the tick was put in a 0.5 ml tube (Axygen) and placed in a -80 °C freezer for long term storage and reference stock. Nymphs were also washed in two sets of de-ionized water and a final wash in 70% ethanol but were not bisected. The other half of the adult tick or up to 5 nymphal ticks were placed into a vial (SARSTEDT) and DNAzol® Direct. One hundred microliters of DNAzol® Direct sample processing reagent was used for each (100 µl/10mg) tick tissue mass. No ticks required more than 100 µl of DNAzol®. Each tick and DNAzol® sample was heated at 80-90 °C for fifteen minutes which allowed tick components to loosen and break up easier (Salazar 2015). After heating, twenty 1.0 mm diameter zirconia/silica beads (BioSpec Products) and two 2.3 mm diameter zirconia/silica beads (BioSpec Products) were added to the tick half (or nymphs) and the DNAzol®. Tick halves (or nymphs), DNAzol®, and zirconia/silica beads were placed in a Mini-Beadbeater-16 (BioSpec Products) for two consecutive three minute cycles at medium speed. Bead-beating allowed tick components to break apart extract the DNA. After the conclusion of the bead-beating, the tubes were centrifuged for one minute at 12,000 RPG in a Legend Micro 21R centrifuge (Thermo Scientific). The resulting liquid was pipetted into a fresh 0.5 ml tube (Axygen) and stored at -20° C until DNA testing (Salazar 2015).

Prior to PCR testing, pools of DNA were created from the extracted samples detailed above. DNA from all adult ticks collected and processed were pooled in groups of 5 for pathogen testing; nymphs were pooled into groups of 25. Pools were separated by collection month and state park and individual tick species. This consisted of taking 5 µl from each tube, up to 5 tubes of individual tick DNA sample, and putting into one 0.5ml tube (Axygen). Next, the DNA concentration of each pool was measured using a

NanoDrop 2000 Spectrophotometer (Thermo Scientific) and concentration was diluted to 240 ng/µl, if necessary, to prevent false negative results (Overzier et al 2013).

PCR Testing

Pooled samples were then tested for the presence of *Ehrlichia chaffeensis* in the *A. americanum* ticks collected using methods described by Salazar (2015). Briefly, a set of nested primers (Ehrlichia/Anaplasma specific outer primers ECB (Table 5.2) and ECC (Table 5.2) followed by *Ehrlichia chaffeensis* specific internal primers HE1 (Table 5.2) and HE3 (Table 5.2)) first developed by Dawson et al. (1996) was used. The following program was used for the outer primer set (ECB/ECC): initial denaturation at 95°C for 5 minutes continued with 30 cycles of 94°C for 1 minute, 65°C for 1 minute, 72°C for 1 minute and a final 72°C for 5 minutes and a holding temperature of 4°C. The program used for the inner primer set (HE1/HE3): initial denaturation at 95°C for 5 minutes continued with 30 cycles of 94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute and a final 72° for 5 minutes and a holding temperature of 4°C.

Pooled samples of *A. americanum* and *D. variabilis* were tested by PCR for *Rickettsia* spp. using the 17kd pan-specific rickettsia primers TZ15 (Table 5.2) and TZ16 (Table 5.2) developed at the CDC (2010). The following program was used: initial 95°C for 5 minutes continued with 30 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a final 72°C for 5 minutes and a holding temperature of 4°C (Salazar 2015).

Ixodes scapularis samples from various state parks were tested by PCR for *Borrelia burgdorferi* using primers ospA2 (Table 5.2) and ospA4 (Table 5.2) developed by Salazar (2015). The program involved: 95°C for 5 minutes continued with 30 cycles

of 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute followed by a final 72°C for 5 minutes and a holding temperature of 4°C (Salazar 2015).

Table 5.2. Primer sets used for PCR assays of pooled tick samples from 6 different state parks in Oklahoma between April and August 2015.

Targeted Gene	Primer Name	Sequence (5' → 3')	Tm (C°)	Fragment Length (bp)		Reference
Ehrlichia chaffeensis 16S rRNA	ECC	AGAACGAACGCTGGCGGCAAGCC	66.8	479	390	Dawson et al. 1996
	ECB	CGTATTACCGCGGCTGCTGGCA	64.6			
	HE1	CAATTGCTTATAACCTTTTGGTTATAAAT	51.6	390		
	HE3	TATAGGTACCGTCATTATCTTCCCTAT	54.3			
Borrelia burgdorferi ospA	ospA2	GTTTTGTAATTTCAACTGCTGACC	53.2	156		Salazar 2015
	ospA4	CTGCAGCTTGGAATTCAGGCACTTC	60.5			
Rickettsia rickettsii 17-kDA antigen	TZ15	TTCTCAATTCGGTAAGGGC	52.3	247		CDC, 2010
	TZ16	ATATTGACCAGTGCTATTTC	42.4			
Tick DNA 16S rRNA	TQ16S +F	CTGCTCAATGATTTTTTAAATTGCTGTGG	56.1	320		Halos et al., 2004; Crowder et al., 2010
	TQ16S -2R	ACGCTGTTATCCCTAGAG	50.6			

Visualization

Gel electrophoresis was used to visualize results of PCR reactions. 1X TBE buffer was used in RunOne™ Electrophoresis Cell (Embi Tec) and ran at 100 V. A 2% agarose gel stained with ethidium bromide was used. Migration time varied between 24-32 minutes for PCR reactions. A 100 base pair ladder was used to determine product size (Life Technologies). Gels were visualized using a White Light/UV transilluminator

(VWR®) at 302 nm and results recorded with a digital CP3800 Cannon camera and printed with a Selphi camera printer.

Verification of Tick DNA

Twenty random tick pools were chosen to verify tick DNA was being used for amplification. The primers used (TQ16S+1F and TQ16S-2R) were described by Halos et al. (2004) and Crowder et al. (2010) and detailed in Table 5.2. The following program used: 95°C for minutes continued with 30 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute followed by a final 72°C for 5 minutes and a holding temperature of 4°C (Salazar 2015).

Assay Protocols using pooled tick samples

No changes in the protocol were made for any of the tick pools. Since all tick pools were measured for DNA concentration and diluted if necessary, 0.5 µl of the tick pool was used as a template for reactions.

Positive Sample Verification

Thirty-one pooled tick samples were randomly chosen and run using the *Rickettsia* and *Ehrlichia* PCR protocols described above. Twenty random tick pools which were positive for Rickettsia 17 kDa (Adults: Roman Nose SP: April pool (P) 1; Lake Thunderbird SP: April P3, May P5, 6, 7, 11, 12, 13, 15, June P3; Sequoyah SP: May P20, 22;, June P3, July P3, August P1Nymphs: Sequoyah SP: Nymph May P3, P5; Aug P1) were sampled and 11 random tick pools for the Ehrlichia PCR protocol (Adults:

Sequoyah SP: May P17, June P4, 5, 9, 12, July P2; Lake Thunderbird SP: April P1, 2, 3; Nymphs: Sequoyah SP June P3, Lake Thunderbird April P4) were sampled (Table 5.5). Five random tick pools (Adults: Sequoyah SP: May P7, 11, 22; Robbers Cave SP: April P2, 3) were sampled for the tick DNA PCR protocol to verify tick DNA (Table 5.5). The positive bands were gel extracted using a PureLink™ Quick Gel Extraction Kit (Invitrogen) and then sequenced at the Oklahoma State University Core Facility. These sequences were then searched in the nucleotide BLAST database to verify the primers amplified the target (Table 5.5).

Analysis of tick data

Because tick DNA was tested by the three PCR assays in pools instead of individually, the maximum likelihood estimation (MLE) was used in Microsoft Excel 2010 (Microsoft Corp, Redmond, WA) to approximate true infection rate of *Rickettsia* spp. and *Ehrlichia chaffeensis* in the field-collected tick samples. This analysis has been used by others who were also focused on tick-borne pathogens in field collected ticks (Nadolny et al 2014; Russart et al 2014). The software used to perform MLE is available from the Centers for Disease Control (CDC, 2013). When conducting the analysis, when all samples are 100% positive, the MLE result is N/A as observed in Table 5.6 & 5.7.

Results

Tick exposure risk indices in state parks across Oklahoma

During the preliminary survey from summer 2014, 755 ticks were collected from 8 state parks (Fig 5.2). The majority of ticks were *A. americanum* (752 (99.6 %)) followed by *D. variabilis* (2 (0.26 %)) and *I. scapularis* (1 (0.013%)).

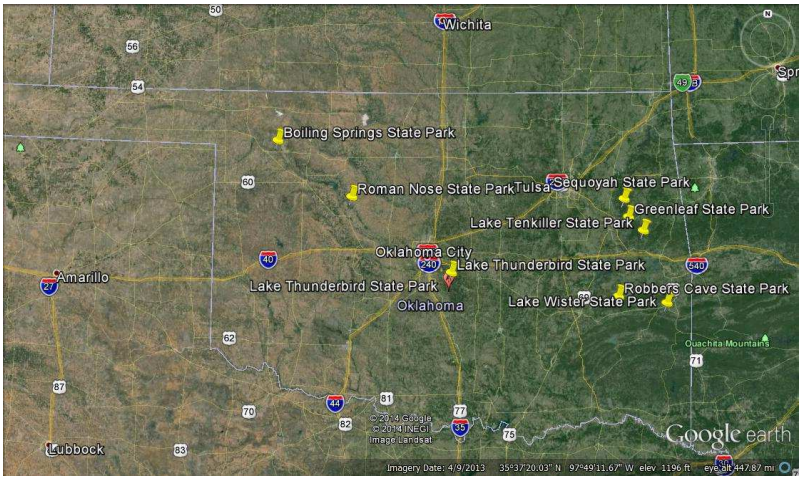

Figure 5.2. Geographic map of state parks sampled in 2014 and 2015. Boiling Springs SP and Lake Tenkiller SP were not revisited in 2015. Credit: Google Earth

During the sampling of summer 2015, 1035 ticks were collected between April and August in 6 state parks: 975 (94.2 %) *A. americanum*, 37 (3.57 %) *D. variabilis* and 23 (2.22 %) *I. scapularis* (Table 5.3). All *D. variabilis*, except two, were collected in Roman Nose SP while *I. scapularis* was collected in three eastern state parks in April and May. In all the eastern and central state parks, between 70% and 82% of all *A. americanum* were collected in May or June while *D. variabilis* was collected between May and July in Roman Nose SP in western Oklahoma. The majority of *I. scapularis* were collected in April in Sequoyah SP, Greenleaf SP and Robber's Cave SP.

Table 5.3. Summary of ticks collected in each state park by species and month in Summer 2015.

State Park	***Month***	***Tick Species***	***Life Stage***	***# of ticks collected (% by park)***
Lake Thunderbird	April	*A. americanum*	Adult	18 (7.69%)
			Nymph	6 (2.56%)
	May	*A. americanum*	Adult	74 (31.6%)
			Nymph	30 (12.8%)
	June	*A. americanum*	Adult	49 (20.9%)
			Nymph	23 (9.83%)
	July	*A. americanum*	Adult	8 (3.42%)
			Nymph	24 (10.25%)
	August	*A. americanum*	Adult	1 (0.43%)
			Nymph	1 (0.43%)
				Total = 234
	July	*D. variabilis*	Adult	2 (100%)
				Total = 2
Roman Nose	April	*D. variabilis*	Adult	3 (8.57%)
	May	*D. variabilis*	Adult	12 (34.3%)
	June	*D. variabilis*	Adult	5 (14.3%)
	July	*D. variabilis*	Adult	13 (52.0%)
	August	*D. variabilis*	Adult	2 (5.71%)
				Total = 35
Sequoyah	April	*A. americanum*	Adult	26 (5.23%)

			Nymph	20 (4.02%)
	May	*A. americanum*	Adult	111 (22.3%)
			Nymph	103 (20.7%)
	June	*A. americanum*	Adult	57 (11.5%)
			Nymph	77 (15.5%)
	July	*A. americanum*	Adult	22 (4.43%)
			Nymph	56 (11.3%)
	August	*A. americanum*	Adult	1 (0.20%)
			Nymph	24 (4.83%)
				Total = 497
	April	*I. scapularis*	Adult	1 (50.0%)
	May	*I. scapularis*	Adult	1 (50.0%)
				Total =2
Greenleaf	April	*A. americanum*	Adult	5 (2.37%)
			Nymph	4 (1.9%)
	May	*A. americanum*	Adult	39 (18.5%)
			Nymph	7 (3.32%)
	June	*A. americanum*	Adult	68 (32.2%)
			Nymph	58 (27.5%)
	July	*A. americanum*	Adult	10 (4.74%)
			Nymph	10 (4.74%)
	August	*A. americanum*	Nymph	10 (4.74%)
				Total = 211

	April	*I. scapularis*	Adult	3 (60.0%)
	May	*I. scapularis*	Adult	2 (40.0%)
				Total = 5
Robbers Cave	April	*A. americanum*	Adult	3 (10.0%)
	May	*A. americanum*	Adult	3 (10.0%)
			Nymph	8 (26.7%)
	June	*A. americanum*	Adult	4 (13.3%)
			Nymph	9 (30.0%)
	July	*A. americanum*	Nymph	3 (10.0%)
	August	no ticks collected		
				Total = 30
	April	*I. scapularis*	Adult	13 (92.8%)
	May	*I. scapularis*	Adult	1 (7.14%)
				Total = 14
Lake Wister	May	*A. americanum*	Adult	1 (33.3%)
	June	no ticks collected/park not available due to flooding		
	July	*A. americanum*	Adult	1 (33.3%)
			Nymph	1 (33.3%)
	August	no ticks collected		
				Total = 3
	April	I. scapularis	Adult	2 (100%)
				Total = 2

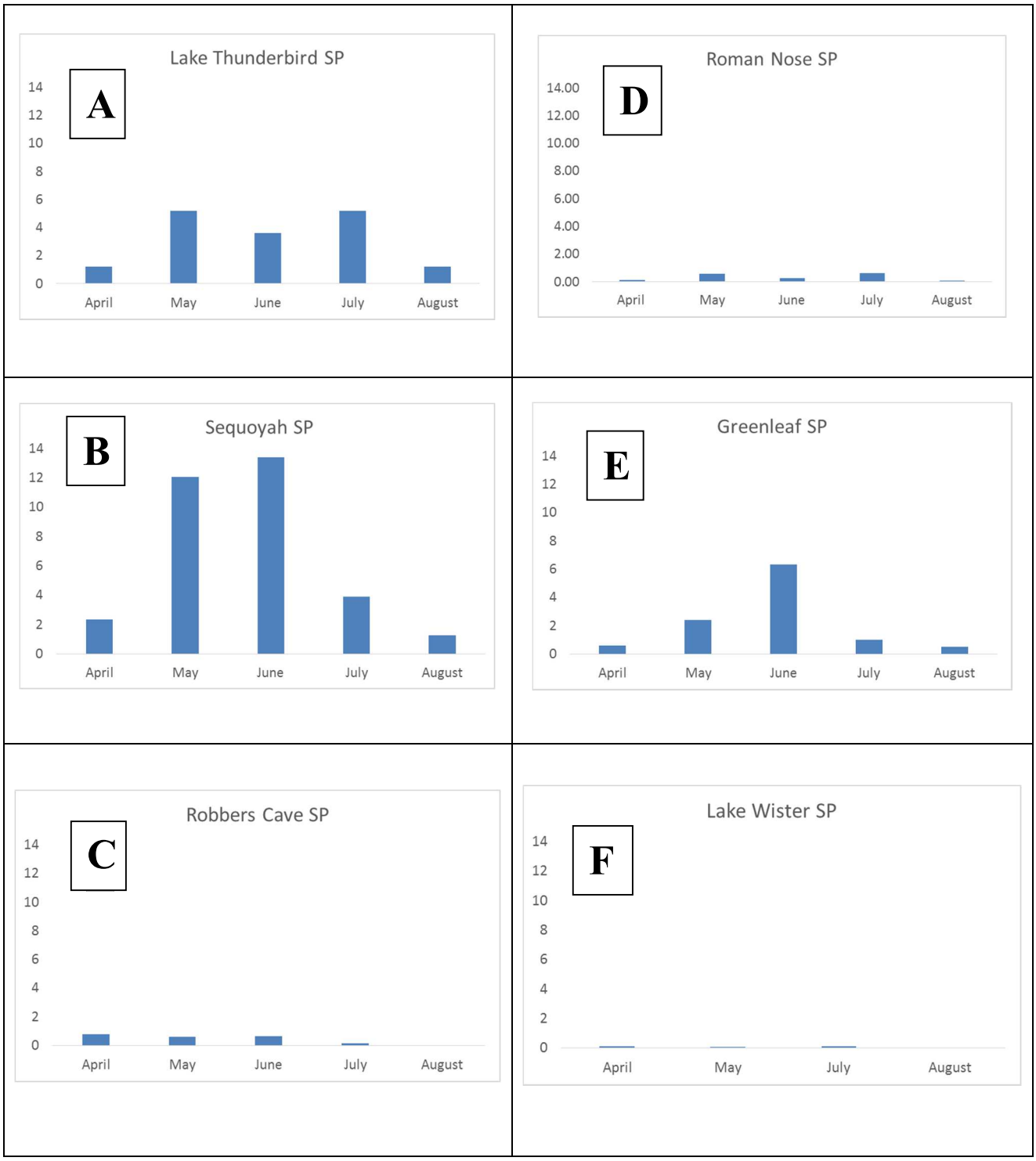

Figure 5.3 A-F. Monthly tick exposure risk (per minute) in 6 state parks across Oklahoma.

Evaluating risk of encountering ticks across state parks in Oklahoma (Fig 5.3 & Table 5.4), it is quite apparent that Sequoyah State Park (Fig 5.3B) has the greatest risk and Lake Wister State Park (Fig 5.3F) has the lowest. This high risk of tick encounter in Sequoyah SP is spread across the months of May and June. Similarly, the second highest tick encounter risk in Lake Thunderbird state park (Fig 5.3A) is spread across three months. Greenleaf State Park (Fig 5.3E), while having a high risk peak in June, has considerably lower encounter risk in May and July. The risk values for Roman Nose (Fig 5.3D), Robbers Cave (Fig 5.3C) and Lake Wister state parks (Fig 5.3F) were all below 1 tick/minute.

Table 5.4 Entomologic risk index in units of ticks/minute for each month at each state park.

State Park	Month	Unit (ticks/minute)	State Park	Month	Unit (ticks/minute)
Lake Thunderbird SP	April	1.2	Greenleaf SP	April	0.6
	May	5.2		May	2.4
	June	3.6		June	6.3
	July	5.2		July	1.0
	August	1.2		August	0.5
Roman Nose SP	April	0.15	Robbers Cave SP	April	0.8
	May	0.6		May	0.6
	June	0.25		June	0.65
	July	0.65		July	0.15
	August	0.1		August	0
Sequoyah SP	April	2.35	Lake Wister SP	April	0.1
	May	12.05		May	0.05
	June	13.4		July	0.1
	July	3.9		August	0
	August	1.25			

Prevalence of tick-borne pathogens in tick pools from Oklahoma state parks

Ehrlichia chaffeensis: Figure 5.4 shows a representative gel used to determine prevalence of *E. chaffeensis* in tick pools collected from 6 state parks (Table 5.6). Total prevalence rates for *E. chaffeensis* from pools of adult and nymphal *A. americanum* from five state parks varied between 0% and 13.9%. Lake Thunderbird (13.9%; 5 positive/36 pools) and Sequoyah SP (13.3%; 8 positive/60 pools) were contrasted with no positive pools from Greenleaf SP (0/33 pools), Robbers Cave (0/6) and Lake Wister (0/2). Comparing total MLE rates was possible only for ticks collected from two state parks. The total MLE for Lake Thunderbird (22.33%; 5 positives/36 pools/228 ticks) was higher than that for Sequoyah SP (16.67%; 8 positives/60 pools/496 ticks). The only state park which had enough individual variability for the MLE to compare infection rates between months was Sequoyah SP with *E. chaffeensis* infections peaking in adult *A. americanum* collected in June 2015. All randomly chosen samples from state parks that were sequenced had sequences which aligned with *E. chaffeensis* (Table 5.5).

Table 5.5. Pools were randomly chosen and positive bands were extracted from the gel and sent to the OSU core facility to be sequenced. Sequences were then placed into BLAST to receive the following information.

Tick Pool	Species	Ident	e- Value	Accession
	***E. chaffeensis* primers**			
Sequoyah SP				
May pool 17	*E. chaffeensis* str. West Paces, complete genome	99%	4e-177	CP007480.1
June pool 4	*E. chaffeensis* str. West Paces, complete genome	99%	7e-180	CP007480.1
June pool 5	*E. chaffeensis* str. West Paces, complete genome	99%	9e-170	CP007480.1
June pool 9	*E. chaffeensis* str. West Paces, complete genome	99%	9e-179	CP007480.1
June pool 12	*E. chaffeensis* str. West Paces, complete genome	99%	1e-177	CP007480.1
June nymph pool 3	*E. chaffeensis* str. West Paces, complete genome	99%	7e-175	CP007480.1
July pool 2	*E. chaffeensis* str. West Paces, complete genome	99%	2e-176	CP007480.1
Lake Thunderbird SP				
April pool 1	*E. chaffeensis* str. West Paces, complete genome	99%	2e-180	CP007480.1
April pool 2	*E. chaffeensis* str. West Paces, complete genome	99%	2e-176	CP007480.1
April pool 3	*E. chaffeensis* str. West Paces, complete genome	99%	3e-179	CP007480.1
April pool 4	*E. chaffeensis* str. West Paces, complete genome	100%	2e-175	CP007480.1
	***Rickettsia spp.* 17kDa primers**			
Roman Nose SP				
April pool 1	*Rickettsia montanensis* str, OSU 85-930, complete genome	99%	1e-100	CP003340.1
Lake Thunderbird SP				
April pool 3	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	2e-103	CP003334.1
May pool 5	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	5e-100	CP003334.1
May pool 6	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	98%	1e-100	CP003334.1
May pool 7	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	98%	3e-82	CP003334.1

May pool 11	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	97%	2e-93	CP003334.1
May pool 12	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	6e-104	CP003334.1
May pool 13	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	98%	8e-98	CP003334.1
May pool 15	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	2e-104	CP003334.1
June pool 3	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	5e-100	CP003334.1
Sequoyah SP				
May pool 20	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	100%	8e-103	CP003334.1
May pool 22	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	100%	4e-101	CP003334.1
May nymph pool 3	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	100%	2e-103	CP003334.1
May nymph pool 5	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	100%	6e-104	CP003334.1
June nymph pool 3	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	8e-103	CP003334.1
July pool 3	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	1e-100	CP003334.1
August pool 1	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	1e-106	CP003334.1
August nymph pool 1	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	8e-103	CP003334.1
Greenleaf SP				
June pool 11	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	2e-104	CP003334.1
June nymph pool 1	Candidatus *Rickettsia amblyommii* str. GAT-30V, complete genome	99%	4e-101	CP003334.1
Tick DNA primers				
May pool 7	*Amblyomma americanum* mitochondrion, complete genome	96%	5e-121	KP941755.1
May pool 11	*Amblyomma americanum* mitochondrion, complete genome	99%	4e-136	KP941755.1
May pool 22	*Amblyomma americanum* mitochondrion, complete genome	99%	2e-118	KP941755.1
Robbers Cave SP				
April pool 3	*Ixodes scapularis* (isolate 2 strain Georgia 3), mitochondrial 16S ribosomal RNA (16S rDNA) gene fragment	91%	6e-105	L43860.1

April pool 2	*Ixodes scapularis* (isolate IscaGA 16S ribosomal RNA gene) partial sequence, mitochondrial	99%	5e-136	EU443405.1

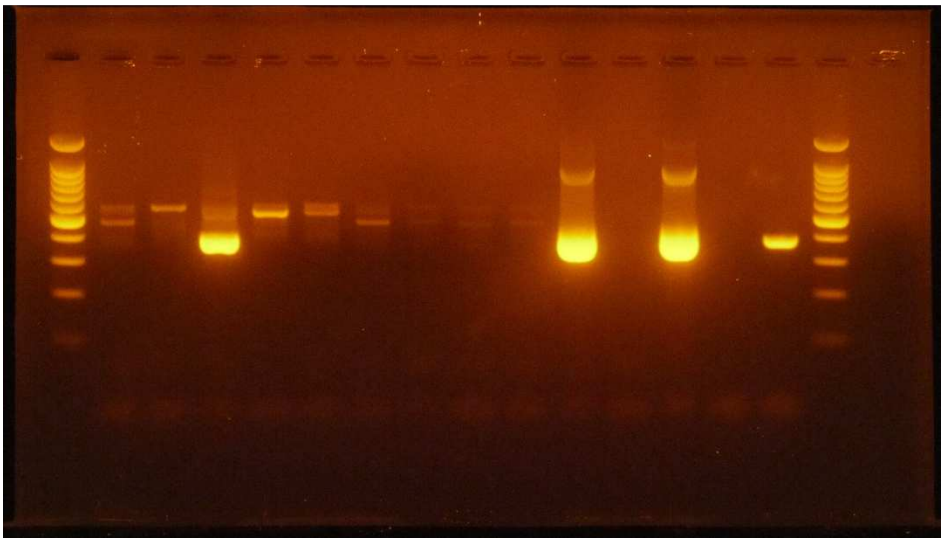

Figure 5.4. Representative gel from the *E. chaffeensis* nested PCR assay on pools of ticks collected in Oklahoma State Parks, nested primers HE1 and HE3, 2% agarose, ran at 100V for up to 32 minutes. Lanes: 1) 100 bp ladder, 2-11) State Park tick pool samples: Seq June nymph Pool 4, Seq July Pool 1, 2, 3, 4, 5, Seq July nymph Pool 1, 2, 3, Seq Aug Pool 1, 12) Negative from ECB/ECC primers, 13) Positive from ECB/ECC primers, 14) Negative from HE1/HE3 primers, 15) Positive from HE1/HE3 primers, 16) 100 bp ladder, 17) blank.

Table 5.6. Prevalence of *Ehrlichia chaffeensis* in pools of Oklahoma state park-collected ticks from summer 2015.

State Park	Month	PCR	Tick Species	Life Stage	Number of positive pools	Number of pools tested	Number of individuals	Positive pools (%)	MLE infection rate (%)
Lake Thunderbird	**April**	Ehrlichia	*A. americanum*	Adults	4	4	18	100	N/A
				Nymphs	0	1	6	0	0
	May	Ehrlichia	*A. americanum*	Adults	1	15	72	6.67	1.39
				Nymphs	0	1	27	0	0
	June	Ehrlichia	*A. americanum*	Adults	0	10	48	0	0
				Nymphs	0	1	23	0	0
	July	Ehrlichia	*A. americanum*	Adults	0	2	32	0	0
				Nymphs	0	1	24	0	0
	August	Ehrlichia	*A. americanum*	Adults	0	1	2	0	0
Sequoyah	**April**	Ehrlichia	*A. americanum*	Adults	0	5	25	0	0
				Nymphs	0	1	20	0	0
	May	Ehrlichia	*A. americanum*	Adults	1	23	111	3.57	0.9
				Nymphs	0	5	103	0	0
	June	Ehrlichia	*A. americanum*	Adults	4	12	57	33	7.68
				Nymphs	1	4	77	25	1.27
	July	Ehrlichia	*A. americanum*	Adults	1	5	22	20	4.56
				Nymphs	0	3	56	0	0
	August	Ehrlichia	*A. americanum*	Adults	1	1	1	100	N/A
				Nymphs	0	1	24	0	0
Greenleaf	**April**	Ehrlichia	*A. americanum*	Adults	0	2	8	0	0
	May	Ehrlichia	*A. americanum*	Adults	0	8	47	0	0
				Nymphs	0	1	7	0	0
	June	Ehrlichia	*A. americanum*	Adults	0	14	68	0	0
				Nymphs	0	3	58	0	0
	July	Ehrlichia	*A. americanum*	Adults	0	3	20	0	0
				Nymphs	0	1	10	0	0
	August	Ehrlichia	*A. americanum*	Nymphs	0	1	10	0	0
Robbers Cave	**April**	Ehrlichia	*A. americanum*	Adults	0	1	2	0	0
	May	Ehrlichia	*A. americanum*	Adults	0	1	3	0	0
				Nymphs	0	1	8	0	0
	June	Ehrlichia	*A. americanum*	Adults	0	1	4	0	0
				Nymphs	0	1	9	0	0
	July	Ehrlichia	*A. americanum*	Nymphs	0	1	3	0	0
Lake Wister	**July**	Ehrlichia	*A. americanum*	Adults	0	2	2	0	0

***Rickettsia* spp.:** Figure 5.5 shows a representative gel use to determine prevalence of *Rickettsia sp.* in tick pools collected from 6 state parks (Table 5.7). Total prevalence rates for *Rickettsia sp.* from pools of adult and nymphal *A. americanum* from five state parks varied between 0% and 93.3%. Sequoyah SP (93.3%; 56 positive/60 pools) had the highest pooled prevalence rate followed by Greenleaf SP (65.5%; 21 positive/32 pools), Robbers Cave SP (50%; 3 positive/6 pools), Lake Thunderbird (47.2%; 17 positive/36 pools), Roman Nose SP (22.2%; 2 positive/9 pools) and Lake Wister SP (0

positive/2pools). The only two state parks which had enough variability for the MLE to compare total infection rates were Lake Thunderbird SP (80.60%; 16 positives/35 pools/226 ticks) and Roman Nose SP (58.37%; 2 positives/9 pools/34 ticks). The *Rickettsia* spp. detected in *D. variabilis* collected from Roman Nose SP were aligned with *R. montanensis* while *Rickettsia sp.* detected in *A. americanum* from all the other State Parks aligned with Candidatus '*R. amblyommii*' (Table 5.5).

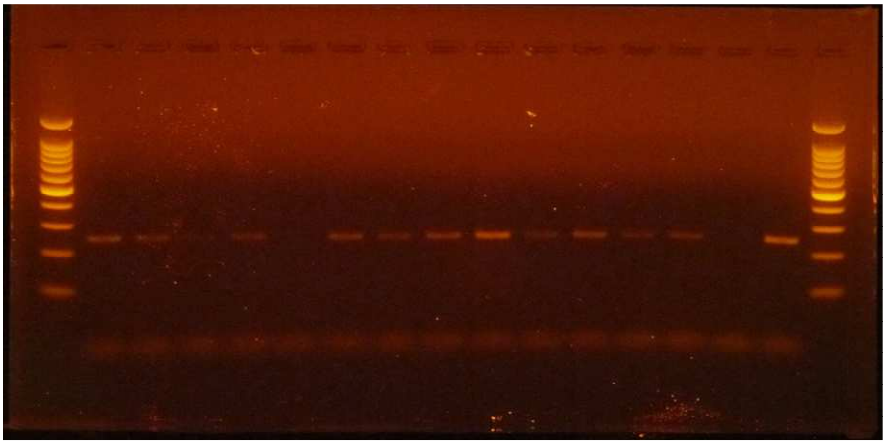

Figure 5.5. Representative gel from the pan-specific *Rickettsia* 17kDa PCR assay on pools of ticks collected in Oklahoma State Parks, primers TZ15 and TZ16, 2% agarose, ran at 100V for up to 30 minutes. Lanes: 1) 100 bp ladder, 2-14) State Park tick pool samples: Sequoyah SP: Adults: May Pool 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, Nymphs: May Pool 1, 15) Negative control, 16) Positive control, and 17) 100 bp ladder.

Table 5.7. Prevalence of *Rickettsia* sp. in pools of Oklahoma state park collected ticks from summer 2015.

State Park	Month	PCR	Tick Species	Life Stage	Number of positive pools	Number of pools tested	Number of individuals	Positive pools (%)	MLE infection rate (%)
Lake Thunderbird	**April**	Rickettsia	*A. americanum*	Adults	0	4	18	0	0
				Nymphs	0	1	6	0	0
	May	Rickettsia	*A. americanum*	Adults	9	15	72	56	16.46
				Nymphs	0	1	27	0	0
	June	Rickettsia	*A. americanum*	Adults	6	10	48	60	16.73
				Nymphs	0	1	23	0	0
	July	Rickettsia	*A. americanum*	Adults	1	2	8	50	10.74
				Nymphs	0	1	24	0	0
		Rickettsia	*D. variabilis*	Adults	0	1	1	0	0
	August	Rickettsia	*A. americanum*	Adults	1	1	2	100	N/A
Roman Nose	**April**	Rickettsia	*D. variabilis*	Adults	1	1	3	100	N/A
	May	Rickettsia	*D. variabilis*	Adults	0	3	12	0	0
	June	Rickettsia	*D. variabilis*	Adults	0	1	5	0	0
	July	Rickettsia	*D. variabilis*	Adults	0	3	12	0	0
	August	Rickettsia	*D. variabilis*	Adults	1	1	2	100	N/A
Sequoyah	**April**	Rickettsia	*A. americanum*	Adults	5	5	25	100	N/A
				Nymphs	1	1	29	100	N/A
	May	Rickettsia	*A. americanum*	Adults	20	23	111	86.9	32.79
				Nymphs	5	5	103	100	N/A
	June	Rickettsia	*A. americanum*	Adults	11	12	57	91.7	36.15
				Nymphs	4	4	77	100	N/A
	July	Rickettsia	*A. americanum*	Adults	5	5	22	100	N/A
				Nymphs	3	3	56	100	N/A
	August	Rickettsia	*A. americanum*	Adults	1	1	1	100	N/A
				Nymphs	1	1	24	100	N/A
Greenleaf	**April**	Rickettsia	*A. americanum*	Adults	1	1	4	100	N/A
	May	Rickettsia	*A. americanum*	Adults	5	8	40	62.5	16.44
				Nymphs	1	1	7	100	N/A
	June	Rickettsia	*A. americanum*	Adults	8	14	68	57.1	15.58
				Nymphs	3	3	58	100	N/A
	July	Rickettsia	*A. americanum*	Adults	1	3	12	33.3	8.44
				Nymphs	1	1	10	100	N/A
	August	Rickettsia	*A. americanum*	Nymphs	1	1	10	100	N/A
Robbers Cave	**April**	Rickettsia	*A. americanum*	Adults	1	1	2	100	N/A
	May	Rickettsia	*A. americanum*	Adults	1	1	3	100	N/A
				Nymphs	1	1	8	100	N/A
	June	Rickettsia	*A. americanum*	Adults	0	1	4	0	0
				Nymphs	0	1	9	0	0
	July	Rickettsia	*A. americanum*	Nymphs	0	1	3	0	0
Lake Wister	**July**	Rickettsia	*A. americanum*	Adults	0	2	2	0	0

***Borrelia burgdorferi*:** No *Borrelia burgdorferi* was detected in 5 pools of 18 *I. scapularis* collected from three state parks (Fig 5.6 & Table 5.8).

Table 5.8. Prevalence of *Borrelia burgdorferi* in pools of *I. scapularis* collected in Oklahoma state parks during Summer 2015.

State Park	Month	PCR	Tick Species	Life Stage	Number of positive pools	Number of pools tested	Number of individuals	Positive pools (%)	MLE infection rate (%)
Sequoyah	**May**	Borrelia	*I scapularis*	Adult	0	1	1	0	0
Greenleaf	**April**	Borrelia	*I scapularis*	Adult	0	1	2	0	0
	May	Borrelia	*I scapularis*	Adult	0	1	2	0	0
Robbers Cave	**April**	Borrelia	*I scapularis*	Adult	0	3	12	0	0
	May	Borrelia	*I scapularis*	Adult	0	1	1	0	0

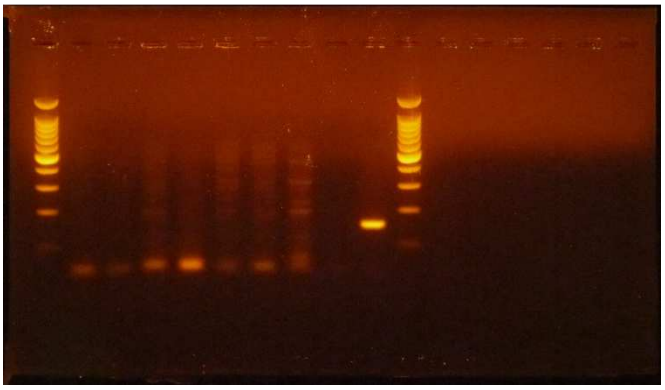

Figure. 5.6. Representative gel from the *B. burgdorferi* PCR assay on pools of ticks collected in Oklahoma State Parks, primers ospA2 and ospA4, 2% agarose, ran at 100V for up to 30 minutes. Lanes: 1) 100 bp ladder, 2-7) State Park pool samples: Sequoyah SP May Pool A, Greenleaf SP: Apr Pool 2, May Pool 9, Robbers Cave SP: Apr Pool 1, 2, 2.5, May Pool 2 8) Negative control 9) Positive control, 10) 100 bp ladder, and 11-17) blank.

Analysis of MLE was done to compare between pathogens, state parks, and months of the year of the sampling. Only the MLE for *Rickettsia* sp. significantly differed between adults and nymphs (Table 5.9) and state parks (Table 5.10). No comparisons of *Ehrlichia chaffeensis* infections were significant.

Table 5.9. Statistical analysis using SAS comparing the tick stage and pathogen.

Pathogen	Stage	Minimal Infection Rate	SEMLEPCT	P value
Ehrlichia	Adults	0.9081	0.53744	0.0808
Ehrlichia	Nymphs	0.0794	0.07938	
Rickettsia	Adults	10.2220	3.13588	0.0472
Rickettsia	Nymphs	0.0000	0.00000	

Table 5.10. Statistical analysis using SAS comparing the State Parks and pathogen.

Pathogen	State Park	Minimal Infection Rate	SEMLEPCT	P value
Ehrlichia	Greenleaf	0.0000 a	0.00000	0.1578
Ehrlichia	Lake Thunderbird	0.1986 a	0.19857	
Ehrlichia	Lake Wister	0.0000 a	.	
Ehrlichia	Robbers Cave	0.0000 a	0.00000	
Ehrlichia	Sequoyah	1.6011 a	0.90620	
Rickettsia	Greenleaf	13.4867 b	2.53552	0.0004
Rickettsia	Lake Thunderbird	4.8811 b	2.50484	
Rickettsia	Lake Wister	0.0000 b	.	
Rickettsia	Robbers Cave	0.0000 b	0.00000	
Rickettsia	Roman Nose	0.0000 b	0.00000	
Rickettsia	Sequoyah	34.4700 a	1.68000	

Table 5.11. Statistical analysis using SAS comparing the collection months and pathogen.

Pathogen	Month	Minimal Infection Rate	SEMLEPCT	P value
Ehrlichia	April	0.0000 a	0.00000	
Ehrlichia	May	0.2863 a	0.19303	
Ehrlichia	June	1.1188 a	0.95040	0.5089
Ehrlichia	July	0.5700 a	0.57000	
Ehrlichia	August	0.0000 a	0.00000	
Rickettsia	April	0.0000 a	0.00000	
Rickettsia	May	13.1380 a	6.13740	
Rickettsia	June	9.7800 a	5.25519	0.3144
Rickettsia	July	2.7400 a	1.78638	
Rickettsia	August	.	.	

Discussion

This study provides a report based on systematic collection of the risk of encountering ticks in 6 Oklahoma State parks. The three parks with the highest risk of encountering ticks were Sequoyah SP, Greenleaf SP, and Lake Thunderbird SP with Sequoyah SP having the highest risk of tick exposure of an average of 6.59 ticks per minute per month, the majority of which were *A. americanum*. There have been other studies that 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; Cilek & Olson 2000; Han et al. 2014; Oliver & Howard 1998; Paskewitz et al. 2001). This appears to be the first record for the encounter risk of *A. americanum* in parks in the southcentral United States

between April and August, the prime time in which most people visit state parks with their companion animals.

Considering risk of encountering ticks within various ecosystems, the considerable differences in risk over the summer of 2015 was quite apparent between Sequoyah SP (highest risk) and Lake Wister SP (lowest risk). These two state parks are in two different ecoregions (okatlas.org, 2004) with Sequoyah SP in denser Eastern and Ozark broadleaf forest, which is characterized by a predominantly Oak-Hickory forest with a well-developed understory (fs.fed.us, 2008), and Lake Wister SP in Southeastern and Ouachita mixed forest, which is characterized by a mix of deciduous trees such as Maple and Elm but with 50% cover coming from Pines (fs.fed.us, 2008). While both State park systems support the same species of ticks in similar proportions, the lack of encountering ticks in Lake Wister SP, while still in eastern Oklahoma, is notable. It is possible that the rain (the month of May 2015 being the wettest month on record with an average rainfall across the state of Oklahoma being 14.06" and Lake Wister setting a new pool record that month with 508.35'(NOAA2015)) which affected the region may have changed some of the risk potential. However, both sites were usually sampled within a day of each other so the data is probably quite representative.

While not as low as Lake Wister SP, Robbers Cave SP with its Southeastern mixed forest and prairie parkland also does not appear to create the same habitat as Sequoyah SP for *A. americanum* to thrive. Interestingly, though, the majority of *I. scapularis* collected during the summer of 2015 were encountered in Robbers Cave SP in April, providing some indication that this kind of survey work should probably be done

year round in these parks in order to truly be able to understand the phenology of tick species in various ecoregions.

The differences in tick encounter risk rates between Sequoyah SP and Greenleaf SP were also notable. Located in the same area of eastern Oklahoma and only 50 minutes apart, Sequoyah SP is characterized by oak-hickory, deciduous forest (fs.fed.us, 2008), while Greenleaf SP is mainly characterized by prairie parkland, which is characterized by an intermingled prairie with strips of deciduous trees such as oak-hickory (fs.fed.us, 2008) which creates a more temperate climate. It is obvious that this difference in vegetation, while small, can make a large impact on risk of experiencing a tick during one's tenure in the park in different months of the summer. The differences between parks can also occur due to the different trails used for sampling. The Greenleaf SP trail was better maintained due to its primacy as hiking trail while the Sequoyah SP trail was used most often utilized as a bike trail and hence did not need the same level of attention by park personnel. The differences could make a change in the way that understory and trail edges were maintained, making it more likely to encounter a tick in Sequoyah SP instead of Greenleaf SP.

While lower in risk than Sequoyah SP, Lake Thunderbird SP in central Oklahoma had a high sustained risk of tick encounter throughout the summer. Compared with the vegetation composition of Sequoyah SP, Lake Thunderbird SP is characterized by Great Plains steppe and shrub and prairie parkland which includes tall grasses with cross timbers and intermingled prairie with strips of deciduous trees (including oak hickory) respectively (fs.fed.us, 2008). This means, that while the vegetation may differ between

the two areas, the habitats are still conducive for *A. americanum* ticks which appear to thrive in both state parks.

Finally, in stark contrast with the other five state parks, Roman Nose SP was only characterized by the collection of *D. variabilis* during the survey period. This tick is associated with drier conditions which are present in the vegetation of Great Plains steppe and shrub which includes tall grasses with cross timbers mix (fs.fed.us, 2008). To date, only one *A. americanum* has been collected in Roman Nose SP despite numerous sampling efforts over a two year period (Dr. B Noden, personal communication). While *A. americanum* has been reported in the county in which Roman Nose SP is present (Barrett et al. 2015), the lack of encountering Lone Star ticks on a regular basis in the park was notable and something that should be further developed in the future.

The results from this study can broadly categorize species of ticks within specific ecoregions in the state. *Amblyomma americanum* was the primary tick present for state parks on the eastern side of the state featuring eastern broadleaf forest, Ozark broadleaf forest, and temperate prairie parkland ecoregions. The primary tick present for state parks in the southeastern parks during April -May was *I. scapularis* in the southeastern mixed forest and Ouachita mixed forest ecoregions. Finally, *D. variabilis* was the only tick species collected at Roman Nose SP which is within the western Great Plains steppe and shrub ecoregion.

The *A. americanum* ticks were tested for *Ehrlichia chaffeensis* as it is an important zoonotic tick-borne pathogen that is increasing in humans and companion animals within the United States (Bayles & Allan 2013; Little et al. 2014) *E. chaffeensis* is the bacteria that causes an infection in humans known as Human Monocytic

Ehrlichiosis (Bayles & Allen 2013). It is thought that the increase in *E. chaffeensis* incidence may be due to the increasing geographical range and abundance of *A. americanum* (Heise et al. 2010; Bayles & Allan 2013; Dahlgren et al. 2015). Pools of *A. americanum* tested positive in Sequoyah SP and Lake Thunderbird SP with total MLE infection rates of 16.7% and 22.3%, respectively. These infection rates are well within the region for *E. chaffeensis* infections in field-collected ticks in the state (Dr. Susan Little, personal communication). While notable that *E. chaffeensis* was not detected in ticks collected from other parks, it does not mean that the pathogen is not present. Future studies are needed to follow up this work to confirm this finding.

Both *D. variabilis* and *A. americanum* ticks were tested for *Rickettsia spp*. as they are the primary vector of *Rickettsia rickettsii* (Heise et al 2010) as well as other Spotted Fever rickettsiae in the Spotted Fever group complex (Dahlgren et al. 2015). The detection of *R. montanensis* in *D. variabilis* collected from Roman Nose SP is notable since *R. montanensis* is a known human pathogen (McQuiston et al. 2012) and a commonly associated endosymbiont in *D. variabilis* (Barrett et al. 2014). The high infection rate of Candidatus '*R. amblyommii*' in *A. americanum* was expected as it is commonly found at prevalence rates between 60% and 90% in field-collected ticks in the United States (Nadolny et al. 2014; Dahlgren et al. 2015). Again, more research is needed to verify which *Rickettsia* are responsible for the Rocky Mountain spotted fever often associated with Oklahoma (Openshaw et al. 2010).

None of the *I. scapularis* pools tested were positive for *Borrelia burgdorferi*. Again, this observation was not surprising as Oklahoma is known to be a 'Lyme free'

state due to feeding of the larvae on lizards instead of *B. burgdorferi* infected white-footed mice (Durden et al. 2002; Garvin et al. 2015).

While all attempts were made to maximize the study design so as to collect data from which correlations can be made, several limitations were present which could influence the extent of comparisons between the state parks. First of all, only one collection replicate was carried out in each month at each park. While valuable, this only enables comparisons between parks over the whole summer period instead of by month. Another limitation is the use of pooled ticks to determine possible prevalence by MLE. Due to the numbers of ticks tested and pool sizes, the *Rickettsia spp*. prevalence is too high for the MLE analysis to work well. Also, due to the overabundance of *A. americanum* in most state parks, the pathogen prevalence in other tick species may not be reliable with the need for further focused collections in the future.

Altogether, the data suggest a high risk for encountering *A. americanum* in eastern and central state parks in Oklahoma between April and August. With high tick risk encounter rates in three of the five in eastern and central parks, the data suggests a relatively high risk of encountering a tick with *Rickettsia spp*. with a low risk for *E. chaffeensis*. Western state parks are mainly dominated by *D. variabilis* which have a relatively low prevalence of *R. montanensis*. Altogether, the data can be used by state parks to continue evaluating strategies to reduce risk of tick encounters and identify new ways to inform the public concerning their personal risk while enjoying the state park.

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REFERENCES CITED

Barrett, A., S. E. Little, E. Shaw. 2014. "*Rickettsia amblyommii*" and *R. montanensis* infection in dogs following natural exposure to ticks. Vector Borne Zoonotic Dis. **14**(1): 20-25

Barrett, A W., B. H. Noden, J. M. Gruntmeir, T. Holland, J. R. Mitcham, J. E. Martin, E. M. Johnson, S. E. Little. 2015. County Scale Distribution of *Amblyomma americanum* (Ixodida: Ixodidae) in Oklahoma: Addressing Local Deficits in Tick Maps Based on Passive Reporting. J. Med. Entomol. **52**: 269-273

Bayles, B. R. and B. F. Allan. 2013. Social-ecological factors determine spatial variation in human incidence of tick-borne ehrlichiosis. Epidemiol. Infect. DOI: 10.1017/S0950268813002951

Carroll, J. F., E. Russek-Cohen, J. D. Nichols, J. E. Hines. 1991. Population dynamics of American Dog ticks (Acari: Ixodidae) along park trails. Environ. Entomol. **20**(3): 922-929

CDC, 2010. PCR Procedure Manual, Rickettsial Zoonoses Branch

Cilek, J. E. and M. A. Olson. 2000. Seasonal distribution and abundance of ticks (Acari: Ixodidae) in Northwestern Florida. J. Med. Entomol. **37**(3): 439-444

Crowder, C. D., M. A. Rounds, C. A. Phillipson, J. M. Picuri, H. E. Mathews, J. Halverson, S. E. Schutzer, D. J. Echer, M. W. Eshoo. 2010. Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. Journal of Medical Entomology. **47**: 89-94

Dahlgren, F. S., C. D. Paddock, Y. P. Springer, R. J. Eisen, C. B. Behravesh. 2015. Expanding range of *Amblyomma americanum* and simultaneous changes in the epidemiology of spotted fever group Rickettsiosis in the United States. Am. J. Trop. Med. Hyg. 15-0580

Dawson, J. E., K. L. Biggie, C. K. Warner, K. Cookson, S. Jenkins, J. F. Levine, J. G. Olson. 1996. Polymerase chain reaction evidence of *Ehrlichia chaffeensis*, an etiologic agent of human ehrlichiosis, in dogs from southeast Virginia. AJVR **57**(8): 1175-1179

Durden, L.A., J. H. Oliver Jr., C. W. Banks, G. N. Vogel. 2002. Parasitism of lizards by immature stages of the blacklegged tick, *Ixodes scapularis* (Acari, Ixodidae). Experimental and Applied Acarology. **26**:257-266

http://www.okatlas.org . 2004. Ecoregions in Oklahoma. N.p., July 2004. Web. 01 Nov. 2015. http://www.okatlas.org/okatlas/biotic/ecoregions.htm

Eisen, L, D. Wong, V. Shelus, R. J. Eisen. 2013. What is the risk for exposure to vector-borne pathogens in United States National parks? J. Med. Entomol. **50**(2): 221-230

Fs.fed.us. 2008. Description of the Ecoregions of the United States. N.p., Bailey, Robert. Aug. 2008. Web. 01 Nov. 2015. http://www.fs.fed.us/land/ecosysmgmt

Garvin, S.D., B. H. Noden, J. W. Dillwith, S. F. Fox, M. E. Payton, R. W. Barker. 2015. Sylvatic Infestation of Oklahoma Reptiles with Immature *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. **52**(5): 873-878

Halos, L., T. Jamal, L. Vial, R. Maillard, A. Suau, A. Le Menach, H. Boulouis, M. Vayssier-Taussat. 2004. Determination of an efficient and reliable method for DNA extraction from ticks. Veterinary Research **35**: 709-713

Han, G. S., E. Y. Stromdahl, D. Wong, A. C. Weltman. 2014. Exposure to *Borrelia burgdorferi* and other tick-borne pathogens in Gettysburg National Military Park, south-central Pennsylvania, 2009. Vector-Borne and Zoonotic Diseases. **14**(4): 227-233

Heise, S. R., M. S. Elshahed, S. E. Little. 2010. Bacterial diversity in *Amblyomma americanum* (Acari: Ixodidae) with a focus on members of the genus *Rickettsia*. J. Med. Entomol. **47**(2): 258-268

Lane, R. S. 1996. Risk of Human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. Am. J. Trop. Hyg. **55**(2): 165-173

Little, S. E., M. J. Beall, D. D. Bowman, R. Chandrashekar, J. Stamaris. 2014. Canine infection with *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma spp*., and *Ehrlichia spp*. in the United States, 2010-2012. Parasit. Vectors. **7**:257

McQuiston, J. H., G. Zemtsova, J. Perniciaro, M. Hutson, J. Singleton, W. L. Nicholson, M. L. Levin. 2012. Afebrile spotted fever group rickettsia infection after a bite from a *Dermacentor variabilis* tick infected with *Rickettsia montanensis*. Vector Borne Zoonotic Dis. **12**(12): 1059-1061

Nadolny, R. M., C. L Wright, D. E. Sonenshine, W. L. Hynes, H. D. Gaff. 2014. Ticks and spotted fever group rickettsiae of southeastern Virginia. Ticks and Tick-borne Diseases. **5**(1): 53-57

Newhouse, V. F. 1983. Variations in population density, movement, and Rickettsial infection rates in a local population of *Dermacentor variabilis* (Acarina: Ixodidae) ticks in the Piedmont of Georgia. Environ. Entomol. **12**: 1737-1746

National Oceanic and Atmospheric Administration (NOAA). TSA Webmaster. Sept 10th, 2015. Web access: Nov 11th, 2015. <http://www.srh.noaa.gov/tsa/?n=climo_summary_may2015>

Oliver, J. and J. J. Howard. 1998. Occurrence of *Ixodes scapularis* (Acari: Ixodidae) on a selected segment of the Appalachian Trail. J. Med. Entomol. **35**(1): 54-58

Openshaw, J. J., D. L., Swerdlow, J. W. Krebs, R. C. Holman, E. Mandel, A. Harvey, D. Haberling, R. F. Massung, J. G. McQuiston. 2010. Rocky Mountain Spotted Fever in the United States, 2000-2007: Interpreting Contemporary Increases in Incidence. Am. J. Trop. Med. Hyg. **83**: 174-182

Overzier, E., K. Pfister, I. Herb, M. Mahling, G. Bock Jr., C. Silaghi. 2013. Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in ticks infesting roe deer in southern Germany. Ticks and Tick-borne Dieseases. **4**(4): 320-328

Pardanani, N., T. N. Mather. 2004. Lack of Spatial Autocorrelation in Fine-Scale Distributions of *Ixodes scapularis* (Acari: Ixodidae). J. med. Entomol. **41**(5): 861-864

Paskewitz, S. M., M. Vandermause, E. A. Belongia, J. J. Kazmierczak. 2001. *Ixodes scapularis* (Acari: Ixodidae): Abundance and rate of infection with *Borrelia burgdorferi* in four state parks in Wisconsin. J. Med. Entomol. **38**(1): 33-38

Rawlings, J. A. & G. J. Teltow. 1994. Prevalence of Borrelia (Spirochaetaceae) Spirochetes in Texas ticks. J. Med. Entomol. **31**(2): 297-301

Salazar, J. L. 2015. Detection of tick-borne pathogens in lab reared tick colonies and wild populations. Master's thesis. Oklahoma State University.

Siderelis, C., R. L. Moore, Y. F. Leung, J. W. Smith. 2012. A nationwide production analysis of park attendance in the United States. Journal of Environmental Management. **99**: 18-26

Siikamaki, J. 2011. Contributions of the US state park system to nature recreation. Proceedings of the National Academy of Sciences USA. **108**:14031-14036

Sonenshine, D. E. 1972. Ecology of the American Dog Tick, *Dermacentor variabilis*, in a Study Area in Virginia. 1. Studies on Population Dynamics Using Radioecological Methods. Annals of the Entomological Society of America. **65**(5): 1164-1175

Sonenshine, D. E. 1993. Biology of ticks, vol. II. Oxford University Press

Zimmerman, R. H., G. R. McWherter, S. R. Bleomer. 1988. Medium-Sized Mammal Hosts of *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) at Land between the Lakes, Tennessee, and effects of integrated tick management on host infestations. J. Med. Entomol. **25**(6): 461-466

CHAPTER VI

SUMMARY OF THESIS

Altogether, these projects have filled in important gaps in knowledge in regards to the ecology of Oklahoma ticks of medical and veterinary importance. Chapters 3 and 5 focused on the importance of the habitats in specific ecoregions for the major tick species habitat along with a county by county update of tick distributions. Chapter 4 filled in another important transporter of ticks in the state through a novel large mammalian host. Additionally, the risk of encountering several tick-borne pathogens were covered in chapter 5 to demonstrate their presence is in ticks in various Oklahoma State Parks. All of the new information presented in this thesis serves to fill in critical gaps regarding the disease triangles where tick-borne pathogens are concerned in the Great Plains region. This information is vital to the complete understanding of tick-borne pathogen diseases within the state at an epidemiological level as well providing ideas for regional differences in the United States.

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- Mitcham, JP, Noden, BH. County Scale Distribution of Oklahoma Tick Species of Medical and Veterinary Importance. Presentation at SW Branch ESA meeting, Tulsa, OK (Feb 2015).
- Mitcham, JP, Skinner, D, Johnson, E, Noden, BH. Ticks and tick-borne pathogens from two populations of American Black Bear in Eastern Oklahoma. Presentation at the ESA, Portland, OR (Nov 2014).
- Mitcham, JP, Skinner, D, Johnson, E, Noden, BH. Tick species found on two populations of American black bear (*Ursus americanus*) in eastern Oklahoma. Presentation at Central Plains Society of Mammology, Bull Shoals Field Station (MSU), MO (Oct 2014).
- Skinner, D, Mitcham, J, Noden, B, Starkey, L, Johnson, E, Fairbanks, S, Little, S. Prevalence of tick species and *Ehrlichia* spp. in Oklahoma black bears (*Ursus americanus*). Poster at Summer Research Training Program, Cornell, NY (July 2014).

Publications:

- Barrett, AW, Noden, BH, Gruntmeir, JM, Holland, T, Mitcham, JR, Martin, JE, Johnson, EM, Little, SE. 2015. County scale distribution of *Amblyomma americanum* in Oklahoma: addressing local deficits in tick maps based on passive reporting. Journal of Medical Entomology. 52(2): 269–273.