MOSQUITOES OF URBAN OKLAHOMA AND

THEIR POTENTIAL AS DISEASE VECTORS


By

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Abstract: The mosquito diversity of Oklahoma was last evaluated in a series of surveys conducted between 2003 and 2006 and before that, the previous survey was in 1965. Prior surveys targeted specific questions including the evaluation of West Nile virus. However, a systematic survey of the cities in Oklahoma was not conducted. In this study, mosquito populations were surveyed approximately bi-weekly at six different cities, including four with military bases, in central and southeastern Oklahoma between May and September 2016. Three different traps were used: CDC Mini Light traps with lights removed and baited with dry ice, CDC Gravid traps baited with Bermuda grass-conditioned water, and BG-Sentinel® traps baited with BG lure. A total of 11,980 adult female mosquitoes representing 34 species were collected over 834 trapping events. Mosquito communities differed significantly among trap type, with CDC Mini Light traps collecting the most individuals. Mosquito communities also differed significantly among cities sampled. These results show that urban areas in Oklahoma do not possess a homogenous mosquito community, and each city should be evaluated individually (at least within the same climate region). *Aedes aegypti,* an important disease vector was recorded in Oklahoma for the first time since 1940. Eighty-eight specimens of *Ae. aegypti* were collected in the cities of Altus, Ardmore, Frederick, and Lawton. CDC Mini Light traps were more attractive to *Ae. aegypti* than other two trap types. The most abundant mosquito species: *Ae. albopictus, Ae. sollicitans, Ae. triseriatus*, *Cx. pipiens,* and *Cx. tarsalis* were tested for presence of *Dirolfilaria immitis,* the causative agent for dog heartworm, and other parasitic nematodes. One mosquito pool tested was PCR positive for *D. immitis,* from a *Cx. pipiens* pool. Six other nematodes were collected in pools from *Ae. albopictus.* The *D. immitis* positive pool was collected from Idabel, OK, while four other positive pools were collected from Ardmore, OK, and one from Midwest City, OK. The information generated from this study provides insight into mosquito populations and potential for nematode transmission in Oklahoma. With increases in human populations living in cities, movement of people and global climate change, the results of this study serves as a baseline and informs mosquito management strategies for southern Oklahoma.

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# I. INTRODUCTION

## MOSQUITOES OF URBAN OKLAHOMA AND THEIR POTENTIAL AS DISEASE VECTORS

ABSTRACT. Mosquitoes are among the most dangerous animals on earth because of their ability to transmit an array of disease organisms and viruses. Knowledge on distribution, densities, feeding behavior, and vector competency of mosquitoes is essential to successful management. The goals of this project were to: catalogue populations of urban mosquito populations occurring in exurban/urban areas across Oklahoma, assess vector competency of Oklahoma mosquitoes for *Dirofilaria immitis*, and to discover whether *Aedes aegypti* exists in the state. It is important to know what mosquito vectors are present in exurban/urban environments in Oklahoma as the identification of these species would assist in the development of management strategies for medically important mosquitoes. Documenting *D. immitis* infections among mosquitoes is important from a veterinary perspective, as it is a model pathogenic nematode transmitted by mosquitoes. *Aedes aegypti* is an important vector of several pathogens, including Zika virus, which has recently become of concern in the Americas.

## Mosquito Lifecycle

The details of mosquito lifecycles differ between species. *Aedes albopictus* is a major pathogen vector throughout the world. This species transmits La Crosse virus, dengue, and Chikungunya (Bonizzoni et al. 2013), and is currently found in 69 of 77 Oklahoma counties (Noden et al. 2015a). Due to its range and disease vector implications, it is a good model species of an *Aedes* lifecycle. Under average conditions, *Aedes albopictus* develops from egg to adult in 19.3 days, although this period increases significantly in response to limited food and lower temperatures (Chan 1971b). On average, each female *Ae. albopictus* lays 46 eggs in a suitable location with standing water. It exhibits a proclivity for laying eggs in artificial containers, such as plastic or metal buckets compared with natural tree holes (Del Rosario 1963; Hawley 1988). This container-breeding habit allows *Ae. albopictus* to invade urban centers. Females are able to oviposit on multiple occasions (Hawley 1988). Eggs have a low natural mortality, which was 9.9% in one study (Chan 1971b). Eggs are harmed by high dissolved oxygen contents in water, and hatch best at low-to-moderate oxygen levels (Hien 1975).

Habitat selection can vary among different mosquito genera and species. It is also important to be familiar with habitat preferences of other genera. Many *Culex* species prefer water infused with sewage or rotting vegetation with sewage overflow. In the case of the *Cx. quinquefasciatus* complex, this type of habitat significantly increases *Culex* oviposition (Chaves et al. 2014). In contrast, many *Anopheles* prefer clean, running water (Overgaard et al. 2002). *Aedes* has variety of habitat preferences, but some important vector species, such as *Ae. aegypti* and *Ae. albopictus*, prefer to oviposit in containers (Fader and Juliano 2014). These two species are known to select containers based on factors such as resource quality from rotting vegetation (Fader and Juliano 2014).

Once an aquatic habitat for oviposition is selected, the eggs are laid. Then, the female will mate again to fertilize more eggs. The period between a female laying eggs and being able to mate and oviposit again is called the gonotrophic cycle, which varies with air temperature. In *Ae. albopictus,* this cycle takes about 8.41 days at 24°C, whereas it takes about 4.92 days at 30°C. At higher air temperatures, females have more gonotrophic cycles during their lifetimes, with two cycles at 24°C and three cycles at 27°C and 30°C (Goindin et al. 2015). This is important in temperate climate areas with higher temperatures such as Oklahoma, because increases in the potential number of eggs being laid in warm months has implications for increased pathogen transmission. More eggs laid can mean an increase in future mosquito vector populations, which can cause a higher amount of disease transmission.

Eggs hatch into larvae, the next stage in the mosquito lifecycle. Under laboratory conditions of 25°C, the larval period of *Ae. albopictus* is 5 to 10 days (Hawley 1988). Total duration of the *Ae. albopictus* life cycle is about 19 days (Hawley 1988). The length of time adults survive is dependent on access to sugar to sustain them. Females can survive in a wide variety of temperatures and humidity when they have access to sugar. When provided with only water, females survived 5-7 days (Hien 1975). When provided with sugar and water and access to a frequent blood-meal, female *Ae. aegypti* can survive for over 100 days post-eclosion (Noden et al. 2016). This lifespan is important from a disease transmission standpoint, as it shows that females of this species may survive for several months under ideal conditions.

**Mosquito-borne Diseases in the Great Plains**

Oklahoma is part of the Great Plains of North America, which also includes Colorado, Montana, Kansas, Nebraska, South Dakota, North Dakota, New Mexico, Texas, and the Canadian provinces of Alberta, Manitoba, and Saskatchewan (Coupland 1961). Because historical data are lacking on mosquitoes gathered in Oklahoma, studying mosquitoes from geographically similar areas can provide insight on mosquito ecology in this state, as well as to help understand future steps to take in mosquito surveillance.

The Great Plains has large grassland areas and migratory bird populations (Rahmig et al. 2009). Because birds are the main food source for many mosquito species, the bird biomass allows for large mosquito populations and creates potential for disease transmission to humans. For example, the Great Plains region has one of the highest incidence rates of West Nile virus (WNV) of any area in the world (O'Brien and Reiskind 2013; CDC 2013). This may be due to the large number of bird reservoir hosts present.

*Culex tarsalis* is the most common vector of WNV in the Western United States (Bell et al. 2005, Turell et al. 2005, DiMenna et al. 2006, Nielsen et al. 2008). A study to assess *Culex tarsalis* feeding preferences in the plains region of Colorado throughout the year found that *Cx. tarsalis* abundance was highest due to feeding on nestling birds with the highest mosquito numbers in the spring and a peak in the last quarter of May. *Cx. tarsalis* preferred avian hosts across all months, with the most preferred avian being the Mourning Dove (Kent et al. 2009). West Nile virus was most common during summer (Kent et al. 2009). As the season progressed more mosquitoes fed on mammals, though they still preferred birds. This is important from a WNV perspective, as knowing the feeding behavior and hosts of this species may help to map future occurrence of WNV.

There are many pathogens present in the Great Plains Region, and some are transmitted by mosquitoes. An example of a pathogen is the La Crosse virus (LCV), which is transmitted by *Ae. triseriatus* and *Ae. albopictus*, two species documented throughout Oklahoma (Noden et al. 2015b). While *Ae. triseriatus* is mostly a woodland treehole mosquito, *Ae. albopictus* commonly inhabits water containers around human dwellings. LCV, which was first reported in the Upper Midwest and Appalachian regions, was isolated from three of 65 *Ae. albopictus* specimens in Texas (Lambert et al. 2009). This represents a westward expansion of LCV, which is facilitated by movement of invasive vectors into new areas, including possibly Oklahoma. The disease may also be brought to urban environments by using *Ae. triseriatus* as a bridge vector that can cause infection of *Ae. albopictus* from infected birds in the area. While *Ae. albopictus* prefers to feed on mammals, birds also comprise a portion of their diet (Faraji et al. 2014). To assess possible transmission of this pathogen, it is important to monitor this invasive vector in order

Another pathogen that can affect local bird populations is avian malaria, which is caused primarily by *Plasmodium relictum* (Liao et al. 2017). It can cause many symptoms in birds, including a reduced ability to lay eggs and lowered success of rearing young (Asghar et al. 2015). Avian malaria is transmitted by several mosquitoes, most notably *Culex tarsalis*, a common species found in parts of Oklahoma (Janovy et al. 1966). Despite such a high potential to negatively affect bird populations, studies on the Great Plains on this disease are lacking, and little is known about its presence in bird and mosquito populations. In addition to the diseases mentioned, there are other important mosquito-borne diseases that are of concern in Oklahoma.

**Western Equine Encephalitis**

In 1944 there was an explosive outbreak of Western Equine Encephalitis (WEE) centered in Oklahoma, which included 1,326 cases in horses and mules (Reeves et al. 1944). Fourteen species of mosquitoes were tested, and no positives resulted from the techniques used at the time. The authors lamented that the study was conducted too late in the outbreak, and that a more accurate assessment could have occurred if conducted sooner (Reeves et al. 1944). In 1972, a survey of mosquito-borne viruses was carried out in six states, including Oklahoma. During this study 173,074 mosquitoes across 41 species were tested for WEE. Turlock virus (TUR) and unidentified viruses were isolated from mosquitoes collected in Oklahoma (Hayes et al. 1976). In 1994, another outbreak of WEE occurred, this time it affected emu farms within a 25-mile radius of Cordell, OK. While many birds are natural reservoirs of this virus, emus were shown to be highly susceptible to the pathogen, exhibiting symptoms ranging from weakness to death (Randolph et al. 1994).

**Dog Heartworm**

Dog heartworm is a disease caused by the parasitic nematode *Dirofilaria immitis*, and is of concern in Oklahoma. Worldwide, complete larval development of *D. immitis* has been recorded in over 60 species of mosquito (Ludlam et al. 1970). In Oklahoma the rate of heartworm infection for dogs is 2.1%, in comparison to the national average of 1.4% (Bowman et al. 2009). While fifteen species of mosquito across four genera (*Aedes,* *Anopheles, Culex, Psorophora*) have been found to be infected with *D. immitis* in Oklahoma, the main vector of dog heartworm seems to be *Aedes albopictus* (Paras et al. 2014). Prior to the arrival of *Ae. albopictus*, *Ae. trivittatus* was considered to be the main vector (Afolabi et al. 1988).

Dog heartworm is transmitted to mammals, mainly Canidae family canines, through bites of mosquitoes. Mosquitoes become infected with the parasite after feeding on an infected animal and ingesting microfilariae. Heartworms complete their lifecycle in the mammalian host, causing harm in dogs through congestive heart failure (Simón et al. 2009). Though it primarily affects dogs, it also causes acute, life threatening illness in cats (Lister et al. 2008). Human infections also occur by mosquito bites from an infected mosquito (Malik et al. 2016). Humans are dead-end hosts but can become infected and develop benign pulmonary lesions (Simón et al. 2012). Dog heartworm was first discovered in humans in a boy in 1887 in Brazil, where the nematodes had migrated to the left ventricle of his heart (Sakurai et al. 2006). The human aspect of *Dirofilaria* is not well understood, because human infections do not always correlate exactly with canine infections (Simón et al. 2012). For example, infections from *D. repens*, a mostly European species, have been found in humans in areas without a documented case of this species in canines (Simón et al. 2012). The human component of *D. immitis* has not been the focus of research into this pathogen, likely due to the low occurrence of infections.

There are five larval stages of *D. immitis*. The adult nematode primarily lives in the pulmonary arteries (Hoch and Strickland 2008). They are dioecious, and after mating release first stage microfilariae (L1) into the bloodstream. While they circulate in the bloodstream, they can be ingested by female mosquitoes feeding on the host. The microfilariae migrate through the mosquito pharynx and enter the midgut where they remain for 24 hours (Cancrini and Kramer 2001). Microfilariae then migrate to the Malpighian tubules, where they molt twice to reach the L3 stage (Lee et al. 2010). The nematode maturation process in the mosquito can only occur if the ambient temperature is above 14°C (Hoch and Strickland 2008). When mature, L3 larvae migrate to the head of the mosquito in preparation to leave the mosquito and enter the host.

*Dirofilaria immitis* larvae are not injected into a host by a feeding mosquito, but must enter the wound directly while the mosquito is feeding. The microfilariae exit the labellae of a female mosquito and must find their way to the wound, using the hemorrhaged hemolymph of the mosquito to help retain motility during this process (McGreevy et al. 1974). This process is relatively inefficient and 45% of larvae never enter a wound but remain on the skin surface (Ewert and Ho 1967). After the L3 larvae enter the host, they molt twice to reach the L5 stage (Lee et al. 2010) in the host's skeletal muscle tissue 50 to 68 days after initial infection. The adult nematodes then migrate to the host's vascular system where they sexually mature (Hoch and Strickland 2008). Adult *D. immitis* are up to 27-cm long for females and up to 17-cm long for males (Knight 1987). Despite the name "heartworm" there is strong evidence that *D. immitis* does not live in the right ventricle of a living host, and is only found in this location post-mortem (Theis 2005). Instead, studies show that the primary location is in the host pulmonary arteries (Wilcox 1960).

In the United States, canine heartworm is mainly transmitted by mosquitoes in the genera *Anopheles, Psorophora, Culex,* and *Aedes* (Ledesma and Harrington 2011). A survey conducted in Georgia reported that *Ae. albopictus* had the highest *D. immitis* infection rate compared with other mosquito species (Licitra et al. 2010). In Stillwater, OK, *Ae. albopictus* and *Ps. colombiae* had the highest rates of infection and appeared to be the most important vectors in Payne County (Paras et al. 2014). Another study in California using $CO_2$-baited encephalitis vector surveillance (EVS) traps found that *Cx. tarsalis* and *Cx. pipiens* had the highest *D. immitis* infection rates of any species captured, accounting for 67% of all heartworm-positive mosquitoes examined (Huang et al. 2013).

While *D. immitis* is usually of concern when associated with domestic dogs it can also infect coyotes, which can sustain wild populations of this pathogen. A study conducted in rural areas of Oklahoma found a prevalence rate of 6.5% in coyotes (Paras et al. 2012). This number was in line with previous studies conducted in Western states. Studies in Eastern states reported infection rates ranging from 16% to 71% (Custer and Pence 1981; Nelson et al. 2003). A similar study conducted in Kansas reported that 8% of coyotes surveyed were infected with adult heartworms, and that older coyotes were more likely to be infected than younger coyotes (Graham 1975). The low rates of infection found in neighboring states provide evidence that coyotes are an unnatural host for *D. immitis*, and that dogs may be the main reservoir. A similar study conducted on dogs in northeastern Kansas in 1974 reported that 16.7% of dogs surveyed were infected with *D. immitis*, with a strong association of *D. immitis* infections along the eastern part of the surveyed range (Graham 1974). The results of these studies show that western areas in the United States may have a lower infection rate of *D. immitis* in coyotes than Eastern states. It would be interesting to survey the Eastern counties of Oklahoma to make comparisons across the state.

**Zika virus**

Zika virus is a rapidly emerging disease that earned widespread concern in 2016. While it has been recorded since 1947 in Uganda, it only recently became a globally recognized problem (Chang et al. 2016). In 1954, a human Zika virus infection was recorded in Nigeria (Chang et al. 2016). Lanicotti et al. (2008) stated "Historically, Zika has rarely been associated with human disease". Since 2005, Zika virus has spread quickly in the New World. Early reports of epidemics began around 2007, with outbreaks in the South Pacific and Southeast Asia. Prior to April 2015, Zika virus was unknown in Brazil (Ventura et al. 2016). However, in 2015, an outbreak occurred in northern Brazil, starting an epidemic that spread throughout all of northern South America, including all Central America and the Caribbean islands and has moved into Mexico. Cases have also been reported in SE Asia where it is thought the virus will rapidly expand in the coming year. Zika virus is transmitted primarily by *Ae. aegypti*, though it also can be transmitted through sexual contact. Zika virus was first isolated from *Ae. aegypti* in 1967 in Malaysia (Marchette et al. 1969). The World Health Organization issued a global health emergency in response to this virus in 2016. Zika is a *Flavivirus*, family Flaviviridae, the same family as WNV, Yellow Fever, and Japanese Encephalitis (Chang et al. 2016). There are two known distinct lineages of the Zika virus, African and Asian (Duffy et al. 2009). Zika virus is important in its own right, and its spread is a model for future disease outbreaks, especially mosquito-borne ones.

While *Ae. aegypti* is identified as the main vector of this pathogen, Zika virus has been transmitted by *Ae. albopictus* in a laboratory study (Wong et al. 2013). While transmission in the field by *Ae. albopictus* have never been documented, it is important to recognize potential vectors. Additionally, *Ae. unilineatus* and *Ae. vittatus*, two species mainly found in Africa and Southeast Asia, were found to harbor the virus (Diallo et al. 2014). Eighty percent of Zika virus infections do not result in symptoms, though the symptoms that commonly occur are myalgia, edema, vomiting, fever, and rash (Duffy et al. 2009). There is also mounting evidence to support that this disease can cause microcephaly in babies that are born to mothers infected with Zika virus during pregnancy (Chang et al. 2016). Guillian-Barre syndrome, an acute condition which causes death through degradation of respiratory muscles or leaves a patient with lifetime disabilities, is also associated with Zika virus (Cardaso et al. 2015). More studies are needed on this topic, though the possible implications are worthy of note. The presence of *Ae. aegypti*, the only confirmed Zika vector in Oklahoma, would make this state a possible location for an outbreak of this disease.

**West Nile virus**

West Nile virus (WNV) is an important arthropod-borne disease in the United States. Though recognized as a human pathogen since 1937, it did not circulate in the United States until 1999 (Artsob et al. 2009). During the WNV season of 2012, the south-central United States, which includes Oklahoma, had a higher proportion of WNV cases compared with the rest of the country (CDC 2013). For this reason, this disease is particularly important in the state of Oklahoma, where consistent human cases of WNV have been documented every year since 2002 (Johnson et al. 2015). This disease causes long-term neurological symptoms, and current studies are being conducted to determine if mortality of infected individuals has also increased (Johnson et al. 2015). The intensity of WNV outbreaks are also periodic, as nationwide outbreaks occurred in 2003, 2007, and 2012. The 2012 outbreak was one of the largest WNV epidemics ever recorded, and was also the largest epidemic recorded in Oklahoma (Johnson et al. 2015).

WNV is a *Flavivirus* with birds as the reservoir host and *Culex spp.* as the primary vectors. Humans, horses, and other vertebrates are dead-end hosts (Blitvich 2008). WNV is associated with drought conditions, as periods of droughts can draw bird populations to bodies of water where mosquitoes are common (Murray et al. 2011). Birds then help distribute the virus to new areas. Blue jays and robins are known reservoirs (Caffrey et al. 2005). Other bird species are critically affected by the virus. For example, in 2002 in Stillwater, OK, 39 of 120 crows died as a result of WNV, with 56 of 78 marked crows dying the next year (Caffrey et al. 2005). In a 12-month span, 72% of this population died, including 82% of the juveniles.

Important vectors for WNV in Oklahoma include *Culex pipiens, Ae. albopictus,* and *Cx. tarsalis*. *Culex coronator* also has potential to be a WNV vector (Alto et al. 2014), and has been steadily moving north and eastward after being discovered in Texas in 2000 (Gray et al. 2008). It was discovered in Oklahoma in a 2003 survey (Paras et al. 2014, Noden et al. 2015b). While *Cx. coronator* does feed on birds, it prefers to feed on mammals (Alto et al. 2014). However, more eggs are produced when a female feeds on avian blood compared with mammalian blood (Alto et al. 2014). Overall, birds are a better nutritional source for *Culex* mosquitoes than mammals (Richards et al. 2010). The emergence of *Cx. coronator* would add another WNV vector to Oklahoma, and possibly increase transmission rates.

## Mosquitoes in Oklahoma and Surrounding Areas

Mosquito survey data in Oklahoma has historically been neglected. Mosquito diversity was evaluated in Oklahoma in 1965, followed by a 50-year gap before new data were published (Noden et al. 2015b). The survey detailed in Noden et al. (2015b) brought the number of recorded mosquito species in Oklahoma to 62, across 9 genera. The published data in this study is also at least a decade old, as the information was gathered 2003-2006. With the threats of Chikungunya and Zika virus in 2015 and 2016, mosquito species diversity in Oklahoma needs to be documented to determine which arboviruses have competent vectors within the state.

There are 85 mosquito species documented in Texas, a state with a larger, more southern, geographical range than Oklahoma (Texas A&M 2013). Although northern parts of Texas is part of the Great Plains, Texas environments range from coastal to upland. In the continental United States, winter conditions are unsuitable for *Ae. aegypti* activity except for parts of Florida and Texas (Monaghan et al. 2016). There are areas of south Texas that support *Aedes* sp. populations without a dormant period in the winter (Monaghan et al. 2016). Texas is important from an Oklahoma perspective because it harbors mosquitoes that would otherwise not be able to survive Oklahoma winters. These mosquito populations then could reinvade across the Oklahoma border during milder months. For example, Brownsville, TX, is one of the few locations in the country where *Ae. aegypti* and *Ae. albopictus* can breed year round (Uejio et al. 2014).

An extensive study of mosquito habitat preferences, focusing on *Ae. aegypti* and *Ae. albopictus* in south Texas found that these species occupied different habitat niches.

*Aedes albopictus* were more common in coastal habitats, whereas *Ae. aegypti* were more common inland (Champion and Vitek 2014). The areas associated with higher *Ae. aegypti* abundance were those with less vegetation and cover compared with coastal areas. Cemeteries had more mosquitoes than tire shops, but tire shops had significantly more *Ae. aegypti* than cemeteries. *Ae. aegypti* also had a positive correlation with urban areas and with building cover instead of vegetative cover. A study conducted in southern Texas , when *Ae. albopictus* were first starting to become established in the state, found that *Ae. albopictus* were more common in trapping sites away from the Rio Grande River as well as from suburban areas (Womack et al. 1993). In this study it was found that *Ae. aegypti* preferred ovipositing in containers more than *Ae. albopictus* (Womack et al. 1993). Texas surveys as important for mosquito surveys in Oklahoma, since Texas may provide breeding areas for new species to invade the Oklahoma, which would not be able to support a stable population year-round.

**Great Plains Ecology**

The Great Plains are highly fragmented as a result of human development (O'Brien and Reiskind 2014). Changing of grasslands into woodland environments in response to lack of routine fire management results in additional habitats for more species of mosquitoes, potentially increasing arbovirus transmission (O'Brien and Reiskind 2014). The Great Plains already has one of the highest rates of WNV transmission in the United States (CDC 2013). Often, urban areas across the Great Plains have greater tree cover than surrounding rural areas and this coverage could be associated with higher incidence of WNV in urban areas such as in Chicago (Ruiz et al. 2004) Thus, it is likely that clusters of mosquito-borne arboviruses develop in urban centers in the Great Plains (Wimberly et al. 2008).

Because of lack of fire control in grasslands, Eastern Red Cedar, *Juniperus virginiana* is an increasing native plant that is changing the Oklahoma landscape, and may provide habitat for mosquitoes. In 1950, surveys found that 1.5 million acres of Oklahoma were invaded by Eastern Red Cedar. By 1994 this acreage exceeded 6 million (Meneguzzo et al. 2015). In Nebraska, Eastern Red Cedar encroachment has a 2% annual expansion rate each year (Walker and Hoback 2007). Larvae of *Ae. albopictus* this species grew significantly larger when fed *J. virginiana* leaves compared with live oak, *Quercus virginiana,* leaves within a laboratory setting (Reiskind and Zarrabi 2011). The fruits of *J. virginiana* were the most nutritious to larvae and caused the most noticeable increase in growth. Since fruits make up the majority of dry weight litter produced by *J. virginiana*, this can provide a substantial food source for *Ae. albopictus.* In forests, *J. virginiana* outcompetes both post oak (*Quercus stellata*) and blackjack oak (*Quercus marilandica*) in Oklahoma (Hallgren et al. 2013). In particular, *J. virginiana* is encroaching heavily in Central and Western Oklahoma, including Stillwater and Enid (Hallgren et al. 2013). However, the expansion is not limited to Oklahoma, and includes more northern latitudes such as in Nebraska (Cortinas and Spomer 2013). Changing habitats in the Great Plains can have serious public health implications by providing better habitats for mosquitoes that transmit disease.

**Are *Ae. albopictus* really displacing *Ae. aegypti*?**

*Aedes aegypti* was last reported in Oklahoma in 1940 (Rozeboom et al. 1942). One hypothesis for its disappearance is that *Ae. aegypti* was displaced by a better competitor, *Ae. albopictus* (Chan et al. 1971a). *Ae. albopictus* has established itself as a urban and suburban pest across the Americas, Europe, and Africa, while *Ae. aegypti* is restricted to urban domestic areas in mostly tropical regions (Juliano and Lounibos 2005). Local extinctions of *Ae. aegypti* have been observed in the presence of *Ae. albopictus* (Juliano et al. 2004).

One hypothesis is that *Ae. aegypti* favors urban environments more than *Ae. albopictus.* Another study by Reiskind and Lounibos (2012) also found that *Ae. aegypti* were found in higher numbers than *Ae. albopictus* in urban, which were drier and hotter than suburban or rural areas. In a study conducted in Singapore, *Ae. aegypti* populations were spreading more rapidly than those of *Ae. albopictus* due to urbanization in those areas (Chan et al. 1971a). These two species were found to only share habitats 1.6% of the time in rural environments and 7.1% in urban environments (Chan et al. 1971a). Since the percentage of Oklahomans living in urban environments has decreased from 68.0% to 66.2% since 1970, this may be a reason for lack of data for *Ae. aegypti*. On the other hand, the urban population percentage of Texas has increased from 79.7% to 84.7% during the same period as known populations of *Ae. aegypti* also increased (Iowa State University 2016).

Population studies in Florida found a decrease in *Ae. aegypti* populations associated with increases in *Ae. albopictus* populations (O'Meara et al. 1995). Waste tires were the most important breeding sites for both of these species, and mosquitoes were sampled with ovitraps. It was found that after *Ae. albopictus* populations colonized tire dumps, *Ae. aegypti* numbers decreased significantly. In cemeteries where flower vases were used as breeding sites, *Ae. aegypti* were initially the most common, but as *Ae. albopictus* moved into the area, *Ae. aegypti* numbers sharply decreased (O'Meara et al. 1995). On the French island of Mayotte the proportion of sites that contained exclusively *Ae. albopictus* instead of both *Ae. albopictus* and *Ae. aegypti* increased significantly from 2007 to 2010 (Beilhe and Arnoux 2012). This change was not accompanied by an increase of sites populated by *Ae. albopictus*. It is important to note that in the most urbanized location of the study, Mtsapere, Mayotte, *Ae. albopictus* populations gained the least over sites associated with *Ae. aegypti*. In Bermuda, *Ae. aegypti* was considered eradicated in the mid-1960s. However, it was confirmed to be present on the island again in 1997. After *Aedes albopictus* was introduced in the year 2000, the population numbers of *Ae. aegypti* sharply decreased (Kaplan and Kendell 2010).

There is also evidence that these two species prefer different habitats. There is evidence that *Ae. aegypti* eggs are significantly less affected by dry conditions than *Ae. albopictus* eggs (Juliano et al. 2002). *Ae. aegypti* eggs were also found in similar numbers at the beginning and end of the wet season, as compared to *Ae. albopictus,* which had significantly less eggs at the start of the wet season following a dry period (Juliano et al. 2002). This study also suggests that *Ae. aegypti* may fare better in warm, dry areas compared with *Ae. albopictus.* Other field studies have found that *Ae. aegypti* survives in hotter, drier climates relative to *Ae. albopictus* (Mogi et al. 1996, Juliano et al. 2004). It is important to note that these studies have only been conducted in Florida.

Elsewhere in the United States, where hotter and drier areas exist, competition between *Ae. aegypti* and *Ae. albopictus* has not been compared. Oklahoma, a state with a wide range of biomes, can be an ideal location to look for *Ae. aegypti* populations in the drier, southwest areas of the state where mosquito monitoring has never been conducted.

*Ae. albopictus* was not recorded in Oklahoma until 1990 (McHugh 1991). This is long after the last documented collection *Ae. aegypti* in the state (Rozeboom 1942). In 1990 a mosquito surveillance program using ovitraps was conducted across 38 Air Force bases in the United States, including Tinker Air Force Base, OK. In this study there was a marked increase in *A. albopictus* abundance during a period with no *Ae. aegypti* observed. No *Ae. aegypti* specimens were collected from Tinker AFB (McHugh 1991). While *Ae. aegypti* most likely was not displaced by *Ae. albopictus* in Oklahoma, competitive pressure from *Ae. albopictus* may be keeping it from re-establishing. An interesting part of *Ae. albopictus* competition is that a study found both inter- and intra-specific competition among larval *Ae. albopictus* increased their susceptibility to dengue infection (Alto et al. 2008). Vector competence that is increased by competition can have implications on arbovirus transmissions in areas with high competitive pressure.

**Dipteran-Spread Diseases and the Military**

Military forces, domestic and abroad, have historically been exposed to many arthropod-borne diseases. This can vary depending on the geographic location of current conflicts around the world. A model disease is leishmaniasis. Between 1967 and 1998 there were 420 cases of cutaneous leishmaniasis in U.S. service members (Martin et al. 1998). More recently, this parasite has been an issue to military members due to deployments to the Middle East during the 21th century. From January 2003 to November 2004, there were 1,178 cases of leishmaniasis among U.S. service members in Iraq and Kuwait (Schleier et al. 2009). Incidence rates among U.S. service members deployed to Iraq or Afghanistan was also quantified at 2.31 per 1000. This not only harmed the effectiveness of deployed troops, but it also has possible implications as far as service members or government contractors being potential reservoirs for infectious arthropod-borne diseases when they return home.

With the emerging threat of Zika virus in the Caribbean, the United States Military has issued guidance to service members and their families to increase awareness of this disease (Woodson 2016). The Armed Forces Blood Program Office issued guidance to all facilities to refuse blood donations from anybody who has travelled to Mexico, the Caribbean, or Central or South America (Fahie 2016). Another memo released in the same timeframe issued guidance to military medical facilities to be aware and to test for possible presence of Zika virus in individuals showing symptoms and to make sure proper mosquito control measures are being taken at their respective posts (Woodson 2016). Despite alerts and mosquito monitoring, there is no public record of mosquito-borne illnesses being more common in military areas than surrounding areas with similar habitats. At an even more basic level, there are no reports comparing mosquito species diversity on military bases compared with their local communities.

A recent mosquito survey conducted in San Antonio, Texas, discovered high mosquito diversity (McPhatter et al. 2012). Mosquito surveys were conducted extensively from the 1940s through the 1980's because of the large military population in the area (McPhatter et al. 2012). San Antonio, Texas is home to several large military installations, and houses 295,907 service members and their families on these bases (DoD 2016). Forty-one species of mosquito were collected in San Antonio alone, however, *Aedes aegypti* was not collected (McPhatter et al. 2012). Four of the species collected had never been captured in the area despite previous extensive mosquito trapping programs. A similar study conducted in Lubbock, Texas, which has no local military installation, yielded 29 species of mosquitoes over a trapping period of 29 months (Bradford et al. 2008). More studies are needed to assess any relation between military bases and mosquito species richness.

Unfortunately there are no published data on studies surveying the transport of invasive mosquito species that are vectors across the world through military transport. This can potentially be a large area of concern and source of exotic mosquito-borne diseases entering the United States. A study in Kansas sought to examine military surveillance of mosquitoes as required by the Department of Defense. Army, Navy, and Air Force entomology units in the state were contacted directly to obtain mosquito surveillance records, but only the Army and Air Force provided the necessary information (Dawes 2016). There is a lack of information forthcoming from military installations within given communities regarding mosquito populations and potential invasion of exotic mosquito species.

**Mosquitoes in Urban Centers**

As the human population becomes increasingly urbanized, the threat of mosquito-borne diseases in these urban centers becomes a larger issue. Urban areas produce large amounts of water catch basin habitats for production of mosquitoes (Gardner et al. 2013).

As a result of catch basins, mosquitoes in these communities stay close to the catch basin area where they developed, with the surrounding trees and shrubs providing resting places for adult mosquitoes. Falling vegetation in the catch basins provide nutrients for larvae. As most mosquito species in a given urban area rarely venture further than 10 m from edge habitats from where they originated, targeting neighborhoods with high tree and shrub densities with catch basins may help to control urban mosquitoes by removing these breeding sites (Reiskind et al. 2016).

Mosquito population diversity and risk within a given urban area are not uniformly distributed. Recent evidence demonstrates that low-income urban areas of the United States produce higher amounts of mosquitoes than urban areas associated with higher incomes (LaDeau et al. 2013). This was due to artificial containers being widespread in low-income areas. However, these relationships shifted from low-income neighborhoods early in the season to higher socio-economic neighborhoods in the later summer as containers holding water changed between neighborhoods (LaDeau et al. 2013). Another study conducted in Baltimore also found that mosquito production shifts from areas with trash refuse early in the season to watered containers near residences later in the season (Becker et al. 2014). The areas with large amounts of urban decay experienced drying of possible water collection sites as the season wore on. This study also found that overall more mosquitoes were produced in higher-socioeconomic neighborhoods during the peak mosquito season. This can mean that higher class areas with purposely watered containers may be at a higher risk for mosquito-borne pathogen transmission than those with high amounts of urban decay (Becker et al. 2014). It is important to note that urban decay does not necessarily mean that the production of mosquitoes and subsequent disease risk is increased.

### ***Aedes aegypti*** **in Urban Centers**

The main vector of Zika virus is *Ae. aegypti*, which has been associated with many urban mosquito-borne disease outbreaks in the United States, even as far north as New York City (Eisen and Moore 2013). It is recognized as the primary vector of dengue fever and yellow fever in urban areas, and is a vector of the emerging Chikungunya virus (Eisen and Moore 2013). Because it is a container-breeding species, it can be transported as eggs or immatures in water-filled containers such as tires (Gubler 1998). In 1964 a mosquito survey that included over 90,000 sites was successful in discovering *Ae. aegypti* in 10 out of 11 states surveyed, with only Oklahoma not yielding *Ae. aegypti* (Morlan and Tinker 1965). No survey since 1978 has been of such a wide and inclusive scope. A second *Ae. aegypti* survey in 1978 failed to detect any in Oklahoma, Kansas, Missouri, or Kentucky (Service 1978). However, *Ae. aegypti* populations are increasing in the United States, potentially because the *Ae. aegypti* eradication project was halted in 1969 due to lack of funds (Slosek 1989).

In Arizona, *Ae. aegypti* disappeared from the urban landscape for over 40 years, reappearing in the 1990s (Walker et al. 2011). In this arid environment study, *Aedes aegypti* was found to prefer older houses in low-income neighborhoods. This hot, dry climate may have allowed *Ae. aegypti* to persist despite competition from species like *Ae. albopictus*, as has been found in other field studies where *Ae. aegypti* was able to survive in these conditions (Mogi et al. 1996, Juliano et al. 2004). This climate may be similar to areas of southwest Oklahoma.

**Trapping Mosquitoes**

There are many different kinds of traps that have been used to survey mosquitoes. The type of trap used can influence estimates of mosquito diversity and population size. For example, the Biogents (BG) Sentinel Trap (Biogents, Regensburg, Germany) focus on catching *Aedes*, while CDC Gravid Traps are mostly for capturing *Culex* (Li et al. 2016, Lee et al. 2016). One of the main traps used to catch *Aedes* mosquitoes is the BG Sentinel Trap. A study in China found that twice as many *Ae. albopictus* were caught using BG Sentinel Traps compared to CDC Light Traps (Li et al. 2016). A study conducted in northern Florida where BG-Sentinel 2® traps with yeast-generated $CO_2$ and lure were used caught three times more *Ae. aegypti* than CDC Mini Light traps with yeast-generated $CO_2$ (Harwood et al. 2015). With a goal of this study being to collect *Ae. aegypti*, it is important for traps with past records of capturing this species to be used.

In Oklahoma past studies have used several different traps. During a 1997-2000 survey, ovitraps were used exclusively, with the goal of trapping *Ae. albopictus* (Noden et al. 2015b). A survey conducted from 2003-2004 included both oviposition traps and CDC Gravid traps (Noden et al. 2015b). A study in 2014 in Oklahoma used CDC Mini Light traps and BG Sentinel traps (Paras et al. 2014). The CDC Mini Light traps were baited with $CO_2$ to increase attractiveness. In Kansas similar trapping methods have been used. A 2012 Kansas Department of Health study surveyed for mosquitoes using exclusively CDC Mini Light traps baited only with $CO_2$.

## Study Objectives

### Objective I: Differences between Traps and Cities

There are gaps in the current information on mosquitoes in Oklahoma that needs to be addressed by a surveillance project. One objective is to analyze trap preference among different mosquitoes. While different traps have been used across many different studies, there are few data analyzing trap preference among mosquito species, and no tests have been performed in Oklahoma. Also, mosquitoes in urban centers need to be more thoroughly studied in Oklahoma. Because of the importance of mosquitoes in transmitting human diseases, mosquito populations among urban, peri-urban, and rural centers need to be studied. Past studies in Oklahoma have found increased mosquito diversity in urban centers, but did not expand on the differences between cities in Oklahoma (Paras et al. 2014). It is important to fully understand the effects of urban landscapes on mosquitoes.

Oklahoma has a wide range of ecoregions with nine distinct climate zones (Oklahoma Climatological Survey 2014). A study in Brazil found that climate was a strong driver of mosquito abundance, and climate models can be used to model mosquito communities (Wilke et al. 2017). Another study found that climate models can be used as a predictive measure for dengue outbreaks in China (Xu et al. 2017). Surveys in Midwest City, Lawton, Idabel, Altus, Enid, and Ardmore would cover four of these regions. A wide range of climate zones may allow for differing mosquito species communities across Oklahoma cities, and may provide data needed to predict differing mosquito populations across the state.

**Objective II: Search for *Aedes aegypti* in Oklahoma**

Another goal of this study is to search for populations of *Ae. aegypti* in Oklahoma. This important mosquito is a vector of Zika virus, dengue, and chikungunya (Chang et al. 2016). With an increased awareness about Zika virus throughout the Americas, it is important to monitor for populations of this species in Oklahoma. Though it has not been reported in an Oklahoma survey since 1940, the arid area of southwest Oklahoma has never been surveyed for mosquitoes (Rozeboom et al. 1942). Several studies have hypothesized that *Ae. aegypti* prefer hot dry climates over *Ae. albopictus*, and this area would be great for testing this hypothesis in Oklahoma (Juliano et al. 2002).

**Objective III: Search for *Dirofilaria immitis* in Oklahoma mosquitoes**

A final goal is to assess distribution of *Dirofilaria immitis,* the causative agent of dog heartworm, in Oklahoma. While Oklahoma has a relatively high incidence rate of *D. immitis* infections in canine populations (Little et al. 2014), little is known regarding the principle vectors of this important parasite. Historically, the main vectors for *D. immitis* in Oklahoma were considered to be *Culex erraticus* (Afolabi et al. 1989) and *Aedes trivittatus* (Afolabi et al. 1988). This research, however, was only conducted in Stillwater, Oklahoma. In the 1990s, *Ae. albopictus* invaded Oklahoma and soon was found throughout most of the counties in the state (Noden et al. 2015a). Therefore, when an in depth study was recently carried out to identify mosquito vectors of D. immitis in Oklahoma (Paras et al. 2014), *Ae. albopictus* was identified as the most important vector species. However, the scope of the study continued to be only one county surrounding Stillwater, OK. There was a need for a wider study to focus on potential mosquito vectors of filarial worms in the state of Oklahoma.  There is a gap of information that can be remedied by looking at distribution and occurrence of *D. immitis* in Oklahoma mosquito populations in order to tell which mosquitoes carry the pathogen, as well to see if certain cities have higher incidence of *D. immitis*.

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# CHAPTER II

## NEW RECORDS OF *AEDES AEGYPTI* IN SOUTHERN OKLAHOMA, 2016

ABSTRACT. *Aedes aegypti* is an important subtropical vector species and is predicted to have a limited year round distribution in the southern United States. Collection of the species has not been officially verified in Oklahoma since 1940. Adult mosquitoes were collected in 42 sites across 7 different cities in Oklahoma using 3 different mosquito traps between May and September 2016. Between July and September 2016, 88 *Ae. aegypti* adults were collected at 18 different sites in 4 different cities across southern Oklahoma. Centers for Disease Control and Prevention Miniature Light traps baited with CO2 attracted the highest numbers of *Ae. aegypti* individuals compared to Biogents (BG)-Sentinelt traps baited with Biogents (BG)-lure and octenol and Centers for Disease Control and Prevention gravid traps baited with Bermuda grass–infused water. The discovery of *Ae. aegypti* within urban/exurban areas in Oklahoma is important from an ecological as well as a public health perspective.

## Introduction

Zika virus, a mosquito-borne flavivirus related to Yellow Fever, Dengue, and West Nile viruses, was first detected outside of Africa and Southeast Asia in 2007 on Yap Island (Hayes 2009). Since 2013, it has been spreading throughout the Americas, where it is primarily transmitted by *Aedes aegypti* (L.) (Hennessey et al. 2016, Weaver et al. 2016). *Aedes aegypti* is characterized as a mostly tropical species, which is unlikely to thrive in the United States, except in southern Texas and Florida, due to seasonal low temperatures (Monaghan et al. 2016). However, the estimated range for the species includes Oklahoma and Arkansas into the central regions of Kansas and Missouri (CDC 2016).

The last published reports of *Ae. aegypti* in Oklahoma were in 1940, where it was noted to be a fairly common household pest as far north as Stillwater (Rozeboom 1938, 1942). Large-scale surveys conducted in 1964 (Morlan and Tinker 1965) or between 2003 and 2006 did not collect any *Ae. aegypti* individuals in Oklahoma (Paras et al. 2014, Noden et al. 2015). More recently, Hahn et al. (2016) reported *Ae. aegypti* in Oklahoma based on one 1988 report from VectorMap (2016) and 2 reports in 2013 and 2014 (CDC 2015), all of which lack voucher specimens and other pertinent collection information. This study had 2 objectives: to determine whether populations of *Ae. aegypti* exist in Oklahoma, and to compare the efficiency of 3 commercial mosquito traps for the collection of *Ae. aegypti* individuals in Oklahoma.

## Materials and Methods

Adult mosquitoes were collected in six urban/ exurban locations in seven different cities in Oklahoma between May 28, 2016, and September 20, 2016 (Fig. 1). Four cities (Lawton, Ardmore, Midwest City, and Enid) each had 16 sampling events, Ardmore and Idabel had 15 sampling events, and Frederick had one sampling event. Because of reports of the occurrence of *Ae. aegypti* in urban areas (Chan et al. 1971, Womack 1993, Eisen and Moore 2013), sites were selected by proximity to urban centers, reported mosquito activity, location in relation to public centers such as parks, and reduced chances of trap disturbance. Oklahoma State University County Extension agents aided in site selection by reporting mosquito problem areas in the cities. Sampling sites were adjusted when *Ae. aegypti* were detected in order to characterize the approximate distribution of the species in a city.

Surveillance efforts utilized three types of mosquito traps: the Centers for Disease Control and Prevention (CDC) Mini Light traps (Bioquip, Rancho Dominguez, CA), with lights removed and baited with dry ice CO2 released from modified insulated coolers; CDC gravid traps (John W. Hock Company, Gainesville, FL) baited with Bermuda grass–conditioned water; and Biogents (BG)-Sentinelt and BG-Sentinel 2t traps (Biogents, Regensburg, Germany), baited with BG-lure (Biogents) and octenol (Biogents).

Mosquitoes were identified using keys by Darsie and Ward (2005). All mosquitoes were viewed at 4.25x magnification under a stereomicroscope and were identified to species. After identification, all mosquitoes were stored in vials at 20°C, with *Ae.*

*aeg*ypti specimens stored separately. Suspected *Ae. aegypti* specimens were verified by Lisa Coburn of Oklahoma State University and documented with photographs. Voucher specimens were deposited at the Oklahoma State University K.C. Emmerson Entomology Museum.

**Results**

Between May and September 2016, 88 *Ae. aegypti* individuals were collected during 798 trap nights at 284 sample sites. *Aedes aegypti* were collected in traps at 18 different sites in 4 different cities. *Ae. aegypti* were collected at seven of 12 sites within the city limits of Altus and two of six sites in Frederick, Ardmore, and Lawton, OK (Fig. 1). The first collection of *Ae. aegypti* (n = 4) occurred on July 8, 2016, at two of the six sample sites in Altus (Table 1). By August 2016, *Ae. aegypti* (n = 15) had also been collected in Frederick (n = 7) and Ardmore (n = 8). In September, *Ae. aegypti*were collected at 2 of 6 sites in Lawton, OK (n = 2).

**Discussion**

Of the 88 *Ae. aegypti* collected in this study, most (n = 64) were collected from 7 sites in Altus and 2 sites in Frederick (n = 6). Among positive locations in these 2 cities, most *Ae. aegypti* (n = 52) individuals were collected at businesses where tires were stored outside the building, followed by areas near abandoned buildings (n = 10), in a public park surrounded by residences (n = 4), in a hotel parking area (n = 2), in an alley (n = 1), in a wetland adjacent to town (n = 1), and at a residence within the community (n = 1). In Ardmore, *Ae. aegypti* individuals were collected near a drainage area adjacent to the downtown area (n = 13), and the remainder came from a wooded area near the downtown area between a public park and residential area (n = 2). In Lawton, *Ae. aegypti* were collected behind a downtown urban office complex (n = 1) and at a dog park on the edge of the city (n = 1).

Among the three trap types used, most *Ae. aegypti* were caught using dry ice–baited modi- fied CDC Mini Light traps (67%), while the second most were caught with BG-Sentinel traps (21%), and the fewest were caught with gravid traps (12%). The dry ice–baited modified CDC Mini Light traps were significantly better at collecting *Ae. aegypti* than the other traps tested ($X^2 = 45.89$, df = 2, $P < 0.001$). However, the *Ae. aegypti* from Lawton, OK were only collected in two separate gravid traps.

The discovery of *Ae. aegypti* populations within the state of Oklahoma is important from an ecological perspective. This is the first verified and published report of *Ae. aegypti* within Oklahoma since 1940 (Rozeboom 1942). While not detected by recent surveillance efforts, *Ae. aegypti* populations may have been present in the state, according to several unpublished records between 1988 and 2014 recording the species in Oklahoma (central) and Comanche (southwest) Counties. This current surveillance effort was the first to sample mosquitoes in southwest Oklahoma, south of the Wichita Mountains or west of Lawton (Rozeboom 1942). *Aedes aegypti* may have been historically present, but it was never considered necessary to target this species in the region due to the dry, drought-prone nature of the area. Additionally, previous surveillance efforts in Ardmore may not have placed traps within the central urban area where *Ae. aegypti* normally occur and, thus, may have missed populations that may have been present.

In our study, significantly more *Ae. aegypti* were collected using modified CDC Mini Light traps baited with $CO_2$ than the other traps. These results differed from a study conducted in northern Florida, in which BG-Sentinel 2 traps with yeast-generated $CO_2$ and lure caught 33 more *Ae. aegypti* than CDC Mini Light traps with yeast-generated $CO_2$ (Harwood et al. 2015). In the current study, only trap-specific lure was used with the BG-Sentinel 2 traps, while dry ice–generated $CO_2$ was used with the CDC Mini Light traps. This may account for the differing results between studies, but sample sizes are also likely too small for effective comparison. While the use of sticky ovitraps may have improved capture rates (Russell and Ritchie 2004), it is notable that all 3 methods used in this study collected *Ae. aegypti*, as others have reported for urban sites (Reiter et al. 1986, White et al. 2009, Arimoto et al. 2015).

The discovery of *Ae. aegypti* in 4 urban areas in Oklahoma is also important from a public health standpoint. By documenting the presence and distribution of *Ae. aegypti*, public health officials can implement preparedness planning by determining the risk of local vector-borne transmission of Zika, Dengue, and Chikungunya viruses. Prior to 2015, Zika was unknown in the Americas (Ventura et al. 2016), but as of 2016, local transmission is occurring in southern Florida via *Ae. aegypti* (Likos et al. 2016). Neighboring Texas is one of the states in the continental USA that is thought to be able to sustain *Ae. aegypti* breeding populations year-round, providing a potential for sustained virus transmission (Hahn et al. 2016). Therefore, Texas may provide a mosquito source for yearly introduction of *Ae. aegypti* if current populations are not able to survive the winter in Oklahoma. Future studies should evaluate whether *Ae. aegypti* populations are actually able to survive the winter in Oklahoma, or if they are recolonizing each year.

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Figure 1. Sites in Oklahoma where *Aedes aegypti* was collected between July and September 2016. Names include all sites where collections were carried out between May and September 2016. The sites marked with * are the sites where *Ae. aegypti* was collected.

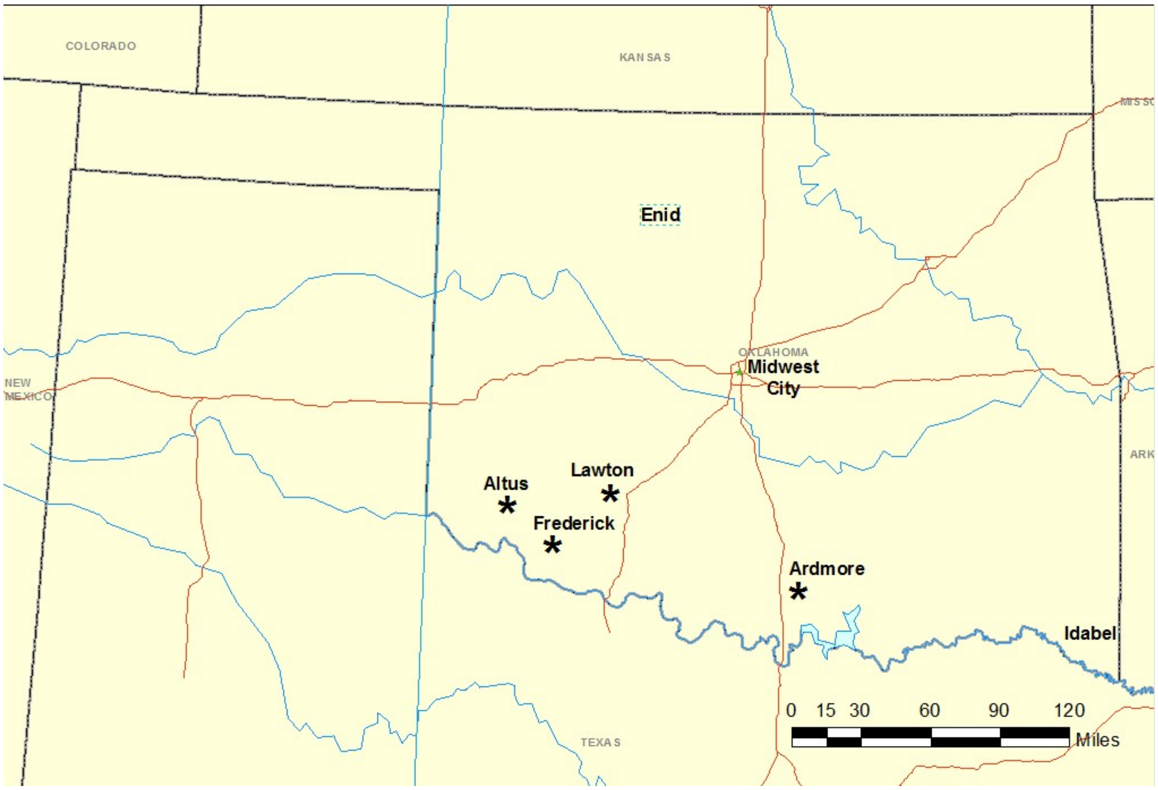

**Table 1.** Numbers of *Aedes aegypti* collected by date and trap type in cities in Oklahoma using three types of traps placed at six sites per location between May and September, 2016.

| City | May | June | July | August | September | TOTAL |
|---|---|---|---|---|---|---|
| Altus | 0 | 0 | 23 | 34 | 7 | 64 |
| Ardmore | 0 | 0 | 0 | 7 | 8 | 15 |
| Enid | 0 | 0 | 0 | 0 | 0 | 0 |
| Frederick* | | | | 7 | | 7 |
| Idabel | 0 | 0 | 0 | 0 | 0 | 0 |
| Lawton | 0 | 0 | 0 | 0 | 2 | 2 |
| Midwest City | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 0 | 0 | 23 | 48 | 17 | 88 |

*Only one sampling was conducted at six locations in Frederick OK using modified CDC Mini Light traps.

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CHAPTER III

# MOSQUITO COMMUNITIES DIFFER AMONG TRAP TYPES AND URBAN AREAS OF OKLAHOMA

ABSTRACT. The mosquito diversity of Oklahoma was last evaluated in a series of surveys conducted from 2003-2006 as part of a West Nile virus surveillance program. Prior to this, the last survey was in 1965. Oklahoma is separated into six ecozones, and cities across these areas may have different mosquito communities. To update the mosquito fauna of Oklahoma, adult female mosquitoes were collected from May to September 2016 using three different traps: CDC Mini Light Traps with lights removed and baited with dry ice $CO_2$ released from modified insulated coolers, CDC Gravid Traps baited with Bermuda grass-conditioned water, and BG-Sentinel® and BG-Sentinel 2® traps baited with BG-lure. These traps were tested to see if they captured different mosquito communities. Traps were set in urban areas associated with 6 cities in central and southeastern Oklahoma, with a focus on cities with military bases. An additional city was added later in the season. 11,980 adult female mosquitoes were collected over 834 trapping events representing 34 species. Mosquito communities significantly differed among trap types with the modified CDC Light traps collecting the most individuals. This study reveals that sampling using multiple trap types across a region is necessary to document mosquito communities for vector monitoring and management programs.

## Introduction

As human populations continue to increase and move to urban areas, monitoring of potential disease vectors in these areas becomes increasingly important. The human population is anticipated to increase by 2.2-billion from 2000 to 2030. Of this increase 2.1 billion will reside in cities (Cohen et al. 2003). Expansion of urban development has been proposed as one of the factors contributing to the increase of mosquito-borne diseases including Dengue and Yellow Fever in many areas (Gubler et al. 2014) where breeding habitats are plentiful and humans comprise the main source of blood meals. In addition to increases in urban human populations, climate change is anticipated to increase the threat of mosquito-borne diseases, especially in temperate zones In fact, mosquito-borne diseases have been documented at high elevations and latitudes for the first time in recent years supporting the idea of an increasing threat (Epstein et al. 1998, Altizer et al. 2013).

Suburban and rural areas can also support bridge vectors, which feed on an infected animal, and then bite and infect a human. Thus, monitoring of suburban areas, especially those located between urban centers, becomes important to monitor for mosquito presence and diversity as well. For example, Brown et al. (2008) found West Nile virus was introduced to the suburban environment of New Haven CT by *Aedes vexans* and then transmitted to humans by *Culex pipiens* which prefer the urban environment.

Peri-urban areas, the areas between expanding urban centers and rural lands, are extremely important for mosquito populations and the diseases transmitted by them. As cities grow, peri-urban areas expand first (Douglas 2006). Most peri-urban areas have poor storm drainage and waste management systems, which provides habitat for mosquito breeding (Parkinson 2003). In particular, *Aedes aegypti* have been documented to quickly occupy new peri-urban areas allowing peri-urban areas to serve as bridges for arboviruses to infect both urban areas and rural areas adjacent to urban centers (Shriram et al. 2008).

Human movement and shipment of materials and supplies may introduce new species and diseases. Military bases are potentially important sources of new diseases to arrive from different areas of the world and military service members have been implicated in introducing infectious diseases into the United States (Mathedo and Davis 2007). Military bases sometimes monitor for mosquitoes as part of vector management programs. However, most of the data remains unpublished and methods often rely on a single type of trap.

This study had two objectives: 1) test the effects of using different trap types on documenting mosquito communities and 2) determine if the mosquito communities associated with 6 urban areas of Oklahoma were similar.

**Materials and Methods**

**Collection of Adult Mosquitoes**

Between May 28, 2016 and September 20, 2016, adult female mosquitoes were collected in six urban/peri-urban locations at each of six different cities in Oklahoma. Four cities (Lawton, Ardmore, Midwest City, and Enid) each had 16 sampling events, Ardmore and Idabel had 15 sampling events. Sites were selected by proximity to urban centers, reported mosquito activity, location in relation to public centers such as parks, and reduced chances of trap disturbance. Oklahoma State University county extension agents aided in site selection by reporting mosquito problem areas in cities. Four of the cities sampled (Lawton, Midwest City, Enid, Altus) contain military bases.

Surveillance efforts utilized three types of mosquito traps: CDC Mini Light Traps (Bioquip, Rancho Dominguez, CA) with lights removed and baited with dry ice $CO_2$ released from modified insulated coolers, CDC Gravid Traps (John W. Hock Company, Gainesville, FL) baited with Bermuda grass-conditioned water, and BG-Sentinel® and BG-Sentinel 2® traps (Biogents, Regensburg, Germany) baited with BG-lure (Biogents) and octenol (Biogents).

Mosquitoes were collected by trap type and location and frozen until identification. All mosquitoes were viewed at 4.25x magnification under a stereomicroscope and were identified to species using Darsie and Ward (2005). After identification, all mosquitoes were stored in 7-dram vials at -20°C. Voucher specimens of all species collected were deposited in the Oklahoma State University insect museum.

**Statistical Analyses**

Analysis of similarity (ANOSIM) was used to measure mosquito species differences among cities and among trap types. ANOSIM is a non-parametric test that analyzes community similarities with ranked dissimilarity rather than raw data (Clarke 1993). ANOSIM produces an associated R-value. High R-values indicate very different groups, while smaller R-values indicate more similar groups. Prior to analysis, data were separated into two groups: early season (May – July), and late season (August – September). ANOSIM was also used to examine mosquito communities by city. The critical alpha value was adjusted using a Bonferroni correction for 15 contrasts per set (0.05/15=0.003) according to Dunnett (1955).

Canonical correspondence analysis (CCA; ter Braak 1986) was used in order to examine differences in mosquito assemblages by city. CCA is a multivariate method used to determine relationships between species and environmental factors affecting their assemblages (ter Braak 1986). All 34 species of mosquitoes collected were used in the analysis and equally weighted.

**Results**

A total of 11,980 mosquitoes were captured across all traps during the surveillance period (Table 1). Thirty-four different species of mosquito that had been previously recorded in Oklahoma were captured among the 834 traps set at 290 sample sites.

Six genera were collected: *Aedes* (n=3,584), *Anopheles* (n=348), *Culiseta* (n=169), *Culex* (n=6,756), *Orthopodomyia* (n=1), and *Psorophora* (n=1,121). *Culex* comprised 56.8% of all mosquitoes captured, while *Aedes* (30.0%) and *Psorophora* (9.3%) were the next most commonly collected genera. Six species comprised 80.8% of all specimens. These were *Cx. pipiens* (32.4%), *Ae. albopictus* (12.0%), *Cx. nigripalpus* (10.3%), *Ps. columbiae* (9.7%), *Ae. sollicitans* (9.5%), and *Ae. triseriatus* (6.9%). Mosquito species with >100 individuals collected were examined for monthly occurrence (Figure 2) and showed differences in activity pattern among species.

**Trap Comparisons**

Because of differences in activity pattern for the most-commonly collected species, early season (May-July) and late-season (August-September) captures were analyzed separately using pairwise contrasts for trap types. Significant differences among all traps during both seasons were observed. The global p-value for both early and late season trapping was 0.001 (Table 2). The global R-statistic was 0.1134 for early season amd decreased in late season to 0.08993. Species with >100 individuals collected were examined for preference to trap type. *An.*

*quadrimaculatus* and *Cx. tarsalis* were significantly more attracted to modified CDC Light traps while *Cx. pipiens* was found significantly less often in BG®-Sentinel traps than others (Table 3).

**Comparisons among Cities**

Mosquito species communities differed among most cities. The city comparison with the highest R-value, indicating the highest difference, was between early season Enid and Altus with an R-value of 0.4096 (Table 4). The cities with the least difference were early season Idabel and Ardmore with an R-value of 0.0175 (Table 4). In early season comparisons, the following cities did not contain significantly different mosquito communities: Idabel and Ardmore, Midwest City and Ardmore, and Lawton and Midwest City (Table 4). In late season the only cities not significant from each other were Ardmore and Midwest City (Table 4).

Mosquito assemblages were also analyzed using CCA. The first CCA model produced three main distinct groups of mosquito diversity (Figure 1). The first CCA axis explained 12.9% of the difference among cities (eigenvalue = 0.452). The second CCA axis explained an additional 7.5% difference (eigenvalue = 0.260). Altus and Idabel were the most different than other cities while the remaining cities had similar mosquito diversity. Three saltmarsh mosquitoes: *Ae. sollicitans, Ae. mitchellae,* and *Ae. dorsalis* that were common only in Altus were removed to create the second model (Figure 2). When these species were removed, Idabel became the most distinct city out of the six sampled (Figure 3).

**Discussion**

Nearly 12,000 adult mosquitoes were collected using three trap types at nearly 36 trapping sites in six urban areas of Oklahoma. No new species were detected in the state, however, a number of previously uncommon species were found in greater than anticipated abundance, including *Ae. aegypti* which had not been observed in Oklahoma in more than 70 years, and salt marsh mosquitoes which had previously been represented by few individuals (Rozeboom 1942; Noden et al. 2015). The most common species encountered was *Cx pipiens*, an invasive species that is important in West Nile Virus transmission (Hesson et al. 2016).

### Comparisons with Previous Surveys

The abundance of *Cx. pipiens* and *Ae. albopictus* was consistent with surveys conducted in 2003, 2004, 2005, and 2006 (Noden et al. 2015). 1,141 *Ae. sollicitans* were captured during the 2016 survey and were 9.0% of all mosquitoes. In the 2003-2005 surveys *Ae. sollicitans* comprised 1.15% of mosquitoes. 95.4% of all *Ae. sollicitans* collected were found in Altus, OK in Jackson County. Due to its semi-arid environment in the southwest portion of Oklahoma, this county has never been surveyed. If the results from Altus, OK would be taken out of the data, *Ae. sollicitans* would comprise 0.5% of the total mosquito species captured. *Aedes sollicitans* is a large and conspicuous species with obvious markings. It is unlikely that it was misidentified in previous surveys. *Ae. triseriatus* were 0.5% of mosquitoes in the 2003-2006 surveys, and were 6.9% of the mosquitoes in the 2016 survey. The differences between this study and previous studies are important to monitor. These data may indicate a shifting mosquito landscape in this area.

### The Saltmarsh Mosquito, *Aedes sollicitans*

*Ae. sollicitans* is a saltmarsh mosquito that is commonly associated with salt plains. It is a competent *D. immitis* vector (Parker 1993) and have tested positive for WNV, Eastern Equine Encephalitis (EEE), and Venezuelan Equine Encephalitis (VEE) (Turell et al. 1992, Molaei et al. 2013). It is a major pest on the Atlantic coast and has been the focus of a number of eradication campaigns (Shone et al. 2006). A female *Ae. sollicitans* can travel up to 110 km (50 miles) from where it eclosed (Ebsary and Crans 1977a). A female can also move 1 mile every day (Ebsary and Crans 1977b). This is a species of concern in Mexico, with few records in the United States (Ortega-Morales 2009).

### *Culex coronator*

*Cx. coronator* is an important disease vector. It is an invasive species in the United States and is originally from Central America (Dyar and Knab 1906). In the United States it has been found in New Mexico, Arizona, and Texas (Darsie and Ward 2005). It was first documented in Oklahoma in 2003, Louisiana in 2005, Alabama in 2007, and South Carolina in 2008 (Debboun et al. 2005 McNelly et al. 2007, Moulis et al. 2008, Noden et al. 2015 ). The Louisiana record was made by a U.S. Army mosquito surveillance program at Fort Polk, Louisiana (Debboun et al. 2005). *Cx. coronator* has been found to be a competent vector for Venezuelan Equine Encephalitis, St. Louis Encephalitis, and West Nile virus in laboratory settings (Aitken et al. 1964, Scherer et al. 1971, Centers for Disease Control and Prevention 2005). In Georgia it is found in old residential areas and hardwood swamps (Moulis et al. 2008). Since *Cx. coronator* have not been widespread in the United States for a long time, their arthropod-borne disease impact is not fully known (Moulis et al. 2008).

This mosquito survey provides evidence that the range of *Cx. coronator* has expanded greatly since the 2003-2006 Oklahoma mosquito surveys. In a 2003 survey, only one female *Cx. coronator* was captured in McAlester, OK (Noden et al. 2015). This latest survey collected 87 additional female *Cx. coronator* in the cities of Lawton, Midwest City, Enid, Altus, Idabel, and Ardmore. Eighty-seven larvae were collected and reared from water collected in a tire pile in

Altus, OK. This species is a possible vector for West Nile virus and has only recently invaded Oklahoma from the southeast (Noden et al. 2015). The widespread distribution of the species recorded in this survey shows that *Cx. coronator* may have expanded its range since the last survey over a decade ago.

**Differences among traps**

Trap type influenced species captures, with modified CDC Light traps capturing significantly more (Table 2) mosquitoes, accounting for approximately 57% of all mosquitoes. Gravid traps that captured 29% and BG sentinel traps that captured approximately 14%. Trap type influenced the capture rate of mosquitoes more strongly in early season samples compared with late-season samples as indicated by the lower R-statistic for late season trapping. The observed differences in trap type indicates that different trap types caught different species of mosquitoes, a result influenced by rarer species. When comparing the most abundantly captured mosquitoes, only three species were observed to significantly differ by trap type (Table 3).

Using only one trap type may not produce an accurate collection of all species found in an area. In this study, CDC Gravid traps which are designed to attract primarily *Culex* mosquitoes had the most captures of *Cx pipiens* (Lee et al. 2016). However, the BG-Sentinel traps designed to attract *Aedes* (Li et al. 2016) did not significantly differ from the other trap types and caught fewer individuals than the modified CDC Light traps (Table 3). In this study, modified CDC Light traps caught the most mosquitoes, however, they were the only traps baited with $CO_2$. A previous study found that CDC Light traps baited with $CO_2$ caught fewer mosquitoes than other light traps (Chen et al. 2011). Future studies should compare the efficacy of other light traps. A study in

Texas found that CDC Light traps with or without lights caught nearly the same amount of mosquitoes (Dennett et al. 2004).

**Differences Among Cities**

Mosquito communities from urban areas of Oklahoma differed with Idabel and Altus being the most different among the six cities surveyed (Figure 1). This result is not unexpected because these cities are the most geographically remote with Altus in the southwest and Idabel in the southeast. However, the results are highly influenced by *Ae. sollicitans, Ae. mitchellae,* and *Ae. dorsalis*, which are three saltmarsh mosquito species which were abundant in Altus. Two of the species, *Ae. mitchellae,* and *Ae. dorsalis* were only collected in Altus in additional to approximately 95% of the *Ae solicitans*. When the salt marsh mosquitoes were removed from analysis, only Idabel differed substantially from the remaining locations (Figure 2). Idabel lies in southeast Oklahoma where annual rainfall is greatest. It is in the the only part of Oklahoma the lies in the Southeastern plains ecosystem that includes much of Louisiana (Environmental Protection Agency). This city also had the most *Ae. albopictus*. This is not surprising, since *Ae. albopictus* has been found to prefer wet areas (Juliano et al. 2002).

Of the cities sampled, Ardmore and Midwest City shared the most similarities in communities and shared the highest observed numbers of *Cx. nigripalpus* and *Ae. triseriatus*. Observed differences in the mosquito community of Lawton are driven by four *Anopheles* species: *An. barberi, An. punctipennis, An. pseudopunctipennis,* and *An. quadrimaculatus,* which were collected most-frequently in that location. The most commonly trapped species, *Cx. pipiens* was captured at similar rates among all cities sampled. The relatively homogenous distribution of this important vector species may be very important to the health of residents in these cities.

The analysis of differences among cities was conducted across the entire season; however, the timing of sampling likely influenced these results as the ANOSIM analyses between cities showed differences between early and late season mosquito species captured. In early season samples, three sets of cities (Lawton and Midwest City, Idabel and Ardmore, and Ardmore and Midwest City) were not statistically different from each other. In late season data, only Midwest City and Ardmore were not statistically different (Table 4). These data show that across the state of Oklahoma, mosquito communities become more differentiated as the season progresses which is also observed in changing abundances of the 10 most commonly collected species across the season (Figure 3). Human occupation of the Great Plains region is highly fragmented which likely influences the establishment and persistence of different mosquito species (O'Brien and Reiskind 2013). Previous studies have also found that closely-related species, such as *Ae. albopictus* and *Ae. aegypti* may prefer different climates. For example, *Ae. aegypti* has been found to prefer hotter and drier areas as compared to *Ae. albopictus* (Juliano et al. 2004). Despite Oklahoma being a large state with many different ecoregions (Oklahoma Forestry Services) because of different climates influenced by precipitation, temperature, and humidity, some mosquito species are widespread (*Cx. pipiens*) while others are localized. The observed differences may be mediated by humans or the environment and future studies are warranted.

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**Figure 1:** CCA model of cities sampled, including all species collected and identified.

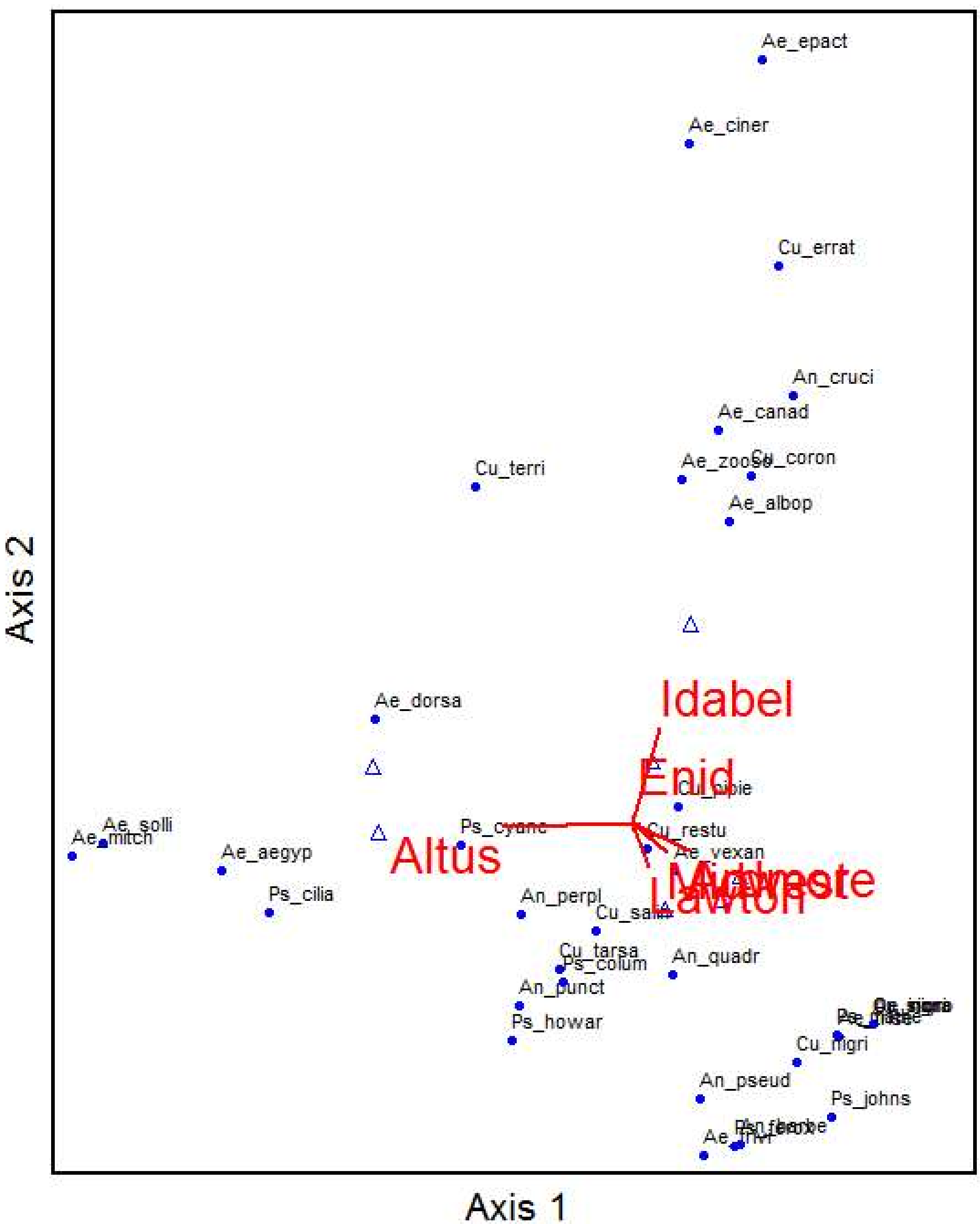

**Figure 2:** CCA model with *Ae. sollicitans, Ae. mitchellae,* and *Ae. dorsalis* removed.

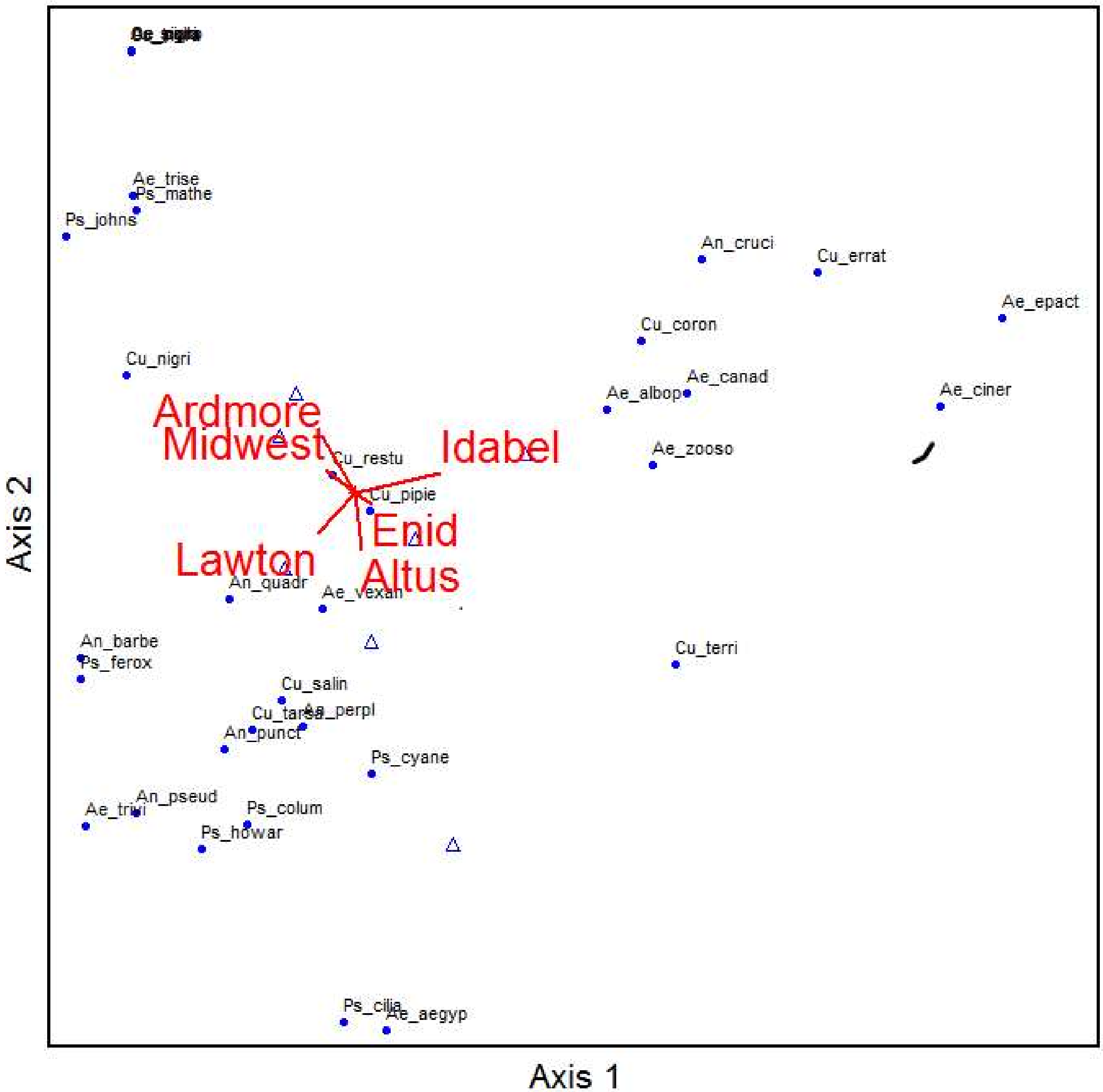

**Figure 3:** Monthly abundance of species with >100 individuals collected.

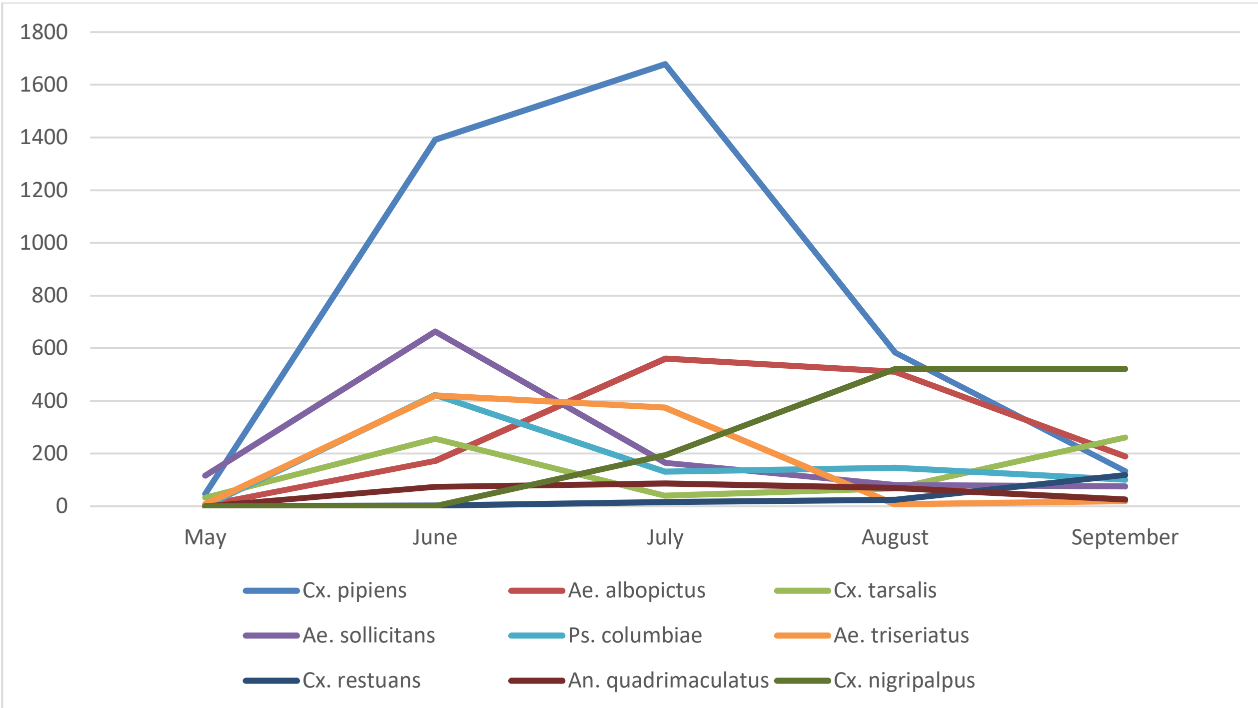

**Table 1.** Collections of adult mosquito across six Oklahoma cities in 2016 using three types of traps. Sampling occurred approximately twice per month between May and September and occurred at six sites within each city.

| Species | City | | | | | | | Trap Type | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Altus | Ardmore | Enid | Idabel | Lawton | Midwest City | Total | Gravid | CDC Light | Sentinel | Total |
| *Aedes aegypti* | 64 | 13 | 0 | 0 | 2 | 0 | **79** | 11 | 52 | 16 | **79** |
| *Ae. albopictus* | 82 | 203 | 226 | 655 | 83 | 181 | **1430** | 457 | 628 | 345 | **1430** |
| *Ae. canadensis* | 1 | 2 | 2 | 7 | 0 | 1 | **13** | 6 | 7 | 0 | **13** |
| *Ae. cinereus* | 2 | 0 | 0 | 17 | 0 | 0 | **19** | 5 | 14 | 0 | **19** |
| *Ae. dorsalis* | 1 | 0 | 1 | 0 | 0 | 0 | **2** | 0 | 2 | 0 | **2** |
| *Ae. epactius* | 0 | 0 | 0 | 1 | 0 | 0 | **1** | 0 | 0 | 1 | **1** |
| *Ae. mitchellae* | 2 | 0 | 0 | 0 | 0 | 0 | **2** | 0 | 2 | 0 | **2** |
| *Ae. sollicitans* | 1087 | 3 | 14 | 20 | 12 | 5 | **1141** | 39 | 697 | 405 | **1141** |
| *Ae. triseriatus* | 0 | 369 | 5 | 30 | 52 | 376 | **832** | 15 | 648 | 169 | **832** |
| *Ae. trivitattus* | 0 | 0 | 0 | 0 | 3 | 0 | **3** | 0 | 3 | 0 | **3** |
| *Ae. vexans* | 6 | 4 | 5 | 8 | 17 | 7 | **47** | 4 | 38 | 5 | **47** |
| *Ae. zoosophus* | 2 | 3 | 3 | 8 | 1 | 0 | **17** | 10 | 7 | 0 | **17** |
| | | | | | | | | | | | |
| *Anopheles barberi* | 0 | 0 | 0 | 0 | 5 | 2 | **7** | 1 | 1 | 5 | **7** |
| *An. crucians* | 0 | 1 | 0 | 4 | 0 | 1 | **6** | 1 | 5 | 0 | **6** |
| *An. perplexens* | 7 | 3 | 2 | 1 | 5 | 2 | **20** | 2 | 15 | 3 | **20** |
| *An. pseudopunctipennis* | 0 | 0 | 1 | 0 | 9 | 0 | **10** | 1 | 0 | 9 | **10** |
| *An. puncticpennis* | 17 | 4 | 2 | 0 | 15 | 10 | **48** | 4 | 38 | 6 | **48** |
| *An. quadrimaculatus* | 34 | 50 | 15 | 18 | 107 | 32 | **256** | 40 | 188 | 28 | **256** |
| | | | | | | | | | | | |
| *Culiseta inoranta* | 0 | 168 | 0 | 0 | 0 | 1 | **169** | 116 | 53 | 0 | **169** |
| | | | | | | | | | | | |
| *Culex coronator* | 5 | 16 | 7 | 42 | 7 | 10 | **87** | 19 | 62 | 6 | **87** |
| *Cx. erraticus* | 4 | 80 | 0 | 306 | 0 | 0 | **390** | 378 | 12 | 0 | **390** |
| *Cx. nigripalpus* | 7 | 531 | 15 | 23 | 435 | 228 | **1239** | 27 | 1198 | 14 | **1239** |
| *Cx. pipiens* | 553 | 948 | 253 | 824 | 936 | 367 | **3881** | 1996 | 1656 | 229 | **3881** |
| *Cx. restuans* | 36 | 54 | 3 | 25 | 21 | 22 | **161** | 30 | 123 | 8 | **161** |
| *Cx. salinarius* | 13 | 2 | 7 | 4 | 20 | 12 | **58** | 10 | 45 | 3 | **58** |
| *Cx. tarsalis* | 185 | 13 | 92 | 6 | 196 | 167 | **659** | 23 | 619 | 17 | **659** |
| *Cx. territans* | 119 | 19 | 1 | 136 | 5 | 1 | **281** | 132 | 149 | 0 | **281** |
| | | | | | | | | | | | |
| *Orthopodomyia signifera* | 0 | 1 | 0 | 0 | 0 | 0 | **1** | 1 | 0 | 0 | **1** |
| | | | | | | | | | | | |
| *Psorophora ciliata* | 36 | 0 | 3 | 0 | 11 | 2 | **52** | 1 | 42 | 9 | **52** |
| *Ps. columbiae* | 195 | 9 | 47 | 49 | 474 | 45 | **819** | 107 | 358 | 354 | **819** |
| *Ps. cyanescens* | 56 | 24 | 26 | 5 | 18 | 1 | **130** | 14 | 98 | 18 | **130** |
| *Ps. ferox* | 0 | 0 | 0 | 0 | 3 | 2 | **5** | 0 | 2 | 3 | **5** |
| *Ps. howardii* | 3 | 1 | 0 | 0 | 5 | 0 | **9** | 0 | 9 | 0 | **9** |
| *Ps. mathesoni* | 0 | 79 | 0 | 2 | 20 | 5 | **106** | 7 | 94 | 5 | **106** |
| **TOTAL** | **2517** | **2600** | **730** | **2191** | **2462** | **1480** | **11980** | **3457** | **6865** | **1658** | **11980** |

**Table 2.** Separation among pairwise communities. Values are ANOSIM *R* (*P*<) pairwise contrasts by trap type.

| | Early Season | | |
|---|---|---|---|
| | gravid | light | sentinel |
| gravid | | 0.0654 (0.001) | 0.2378 (0.001) |
| light | 0.0654 (0.001) | | 0.0816 (0.002) |
| sentinel | 0.2378 (0.001) | 0.0816 (0.002) | |
| | **Late Season** | | |
| | gravid | light | sentinel |
| gravid | | 0.1059 (0.001) | 0.0669 (0.001) |
| light | 0.1059 (0.001) | | 0.0993 (0.001) |
| sentinel | 0.0669 (0.001) | 0.0993 (0.001) | |

**Table 3.** Species with >100 individuals collected compared by trap type. Differing letters indicates a significant difference of p>0.05.

| Species | Gravid | Light | Sentinel |
|---|---|---|---|
| *Anopheles quadrimaculatus* | $0.17±0.06^{A}$ | $0.78±0.16^{B}$ | $0.12±0.05^{A}$ |
| *Culex nigripalpus* | $0.11±0.03^{AB}$ | $3.75±1.65^{A}$ | $0.06±0.03^{B}$ |
| *Culex restuans* | $0.13±0.06^{A}$ | $0.51±0.22^{A}$ | $0.03±0.02^{A}$ |
| *Aedes triseriatus* | $0.05±0.03^{A}$ | $2.45±1.51^{A}$ | $0.70±0.58^{A}$ |
| *Aedes taeniorhynchus* | $0.43±0.17^{A}$ | $1.36±0.40^{A}$ | $1.46±0.79^{A}$ |
| *Aedes sollicitans* | $0.14±0.08^{A}$ | $2.23±1.18^{A}$ | $1.69±1.45^{A}$ |
| *Culex tarsalis* | $0.08±0.02^{A}$ | $1.76±0.71^{B}$ | $0.07±0.03^{A}$ |
| *Culex pipiens* | $7.67±1.74^{A}$ | $5.73±1.18^{A}$ | $0.92±0.27^{B}$ |

**Table 4.** Separation among pairwise communities. Values are ANOSIM *R* (*P*<) pairwise contrasts by city.

| | Early Season | | | | | |
|---|---|---|---|---|---|---|
| | Altus | Ardmore | Enid | Idabel | Lawton | Midwest City |
| Altus | | 0.352 (0.001) | 0.4096 (0.001) | 0.3333 (0.001) | 0.2556 (0.001) | 0.3821 (0.001) |
| Ardmore | 0.352 (0.001) | | 0.2083 (0.002) | 0.0175 (0.229) | 0.0753 (0.025) | 0.0928 (0.074) |
| Enid | 0.4096 (0.001) | 0.2083 (0.002) | | 0.2251 (0.002) | 0.2353 (0.01) | 0.1278 (0.02) |
| Idabel | 0.3333 (0.001) | 0.0175 (0.229) | 0.2251 (0.002) | | 0.1346 (0.007) | 0.2129 (0.005) |
| Lawton | 0.2556 (0.001) | 0.0753 (0.025) | 0.2353 (0.001) | 0.1346 (0.007) | | 0.0519 (0.149) |
| Midwest City | 0.3821 (0.001) | 0.0928 (0.074) | 0.1278 (0.02) | 0.2129 (0.005) | 0.0519 (0.149) | |

| | Late Season | | | | | |
|---|---|---|---|---|---|---|
| | Altus | Ardmore | Enid | Idabel | Lawton | Midwest City |
| Altus | | 0.3603 (0.001) | 0.1656 (0.003) | 0.2782 (0.001) | 0.2098 (0.002) | 0.3698 (0.001) |
| Ardmore | 0.3603 (0.001) | | 0.0991 (0.004) | 0.1302 (0.008) | 0.302 (0.001) | 0.02 (0.164) |
| Enid | 0.1656 (0.003) | 0.0991 (0.004) | | 0.1028 (0.009) | 0.1461 (0.002) | 0.0875 (0.01) |
| Idabel | 0.2782 (0.001) | 0.1302 (0.008) | 0.1028 (0.009) | | 0.2199 (0.001) | 0.147 (0.001) |
| Lawton | 0.2098 (0.002) | 0.302 (0.001) | 0.1461 (0.002) | 0.2199 (0.001) | | 0.3149 (0.001) |
| Midwest City | 0.3698 (0.001) | 0.02 (0.164) | 0.0875 (0.01) | 0.147 (0.001) | 0.3149 (0.001) | |

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CHAPTER IV

# CIRCULATION OF *DIROLFILARIA IMMITIS* AND OTHER PARASITIC NEMATODES IN MOSQUITOES IN SOUTHERN OKLAHOMA

ABSTRACT. Dog heartworm, *Dirofilaria immitis*, is caused by a filarial nematode, and is transmitted by mosquitoes worldwide. During summer 2016, adult mosquitoes were trapped in six urban areas of Oklahoma, using three different trap types: CDC Miniature Light Traps baited with $CO_2$, CDC Gravid Traps baited with Bermuda grass-infused water, and BG Sentinel Traps baited with octenol. A total of 1,227 pools of ≤10 mosquitoes (species including female *Ae. albopictus, Ae. sollicitans, Ae. triseriatus*, *Cx. pipiens, Cx. tarsalis*) were tested for presence of *D. immitis* using polymerase chain reaction (PCR). Seven of these pools (<1%) tested positive for *Dirofilaria* or other nematodes. One of the positive mosquito pools tested had high sequence homology for *D. immitis*. One *Ae. albopictus* pool had a high sequence homology to *Brugia pahangi* and it most likely *B. beaveri*. Five *Ae. albopictus* pools had high sequence identities to *Mansonella ozzardi* and is mostlikely *M. llewellyni*. The *D. immitis* positive pool for was collected from Idabel, OK while four of the *M. ozzardi* and the *B. pahangi* positive pools were collected from Ardmore, OK, and one was from Midwest City, OK. The results of this study offer insight to nematode transmission by mosquitoes associated with urban areas in Oklahoma.

## Introduction

The nematode *Dirofilaria immitis* is an important parasite that often impacts the health of their host and can cause a fatal infections, particularly in cats (Litster et al. 2008). *D. immitis* is also the most important helminthic pathogen that affects dogs in North America, and is found in every state in the United States (Bowman et al. 2009; Little et al. 2014). While *D. immitis* primarily affects canines, humans are dead-end hosts, with rarely fatal results (Theis et al. 2001; Simon et al. 2012). However, up to 80% of humans infected with the parasite are asymptomatic (Miyoshi et al. 2006). Although the parasite has been found to infect many animals, including ferrets, beavers, cats, sea lions, and horses (Otto 1975), the rate of infection is lower. The infection rate of cats is usually 5-20% of the infection rate for dogs in the same area (Atkins et al. 2005).

In North America, vectors of *D. immitis* are continuing to be discovered, though the core group of vectors is known (Ledesma and Harrington 2011).. For example, the invasive *Ae. koreicus* was identified as a competent vector in Europe just in 2015 (Montarsi et al. 2015). One of the most important vectors for *D. immitis* is *Ae. albopictus* which has been implicated in heartworm transmission across the world, including Europe, North America, and Asia (Camprini and Gabrielli 2007, Genchi et al. 2007, Morchón et al. 2012). The expansion of *D. immitis* in Italy was found to match the 1990 introduction of *Ae. albopictus* in that country (Otranto et al. 2013). It feeds mainly on mammals, but is highly attracted to dogs and may play a larger role in *D. immitis* transmission than other species, including *Culex pipiens* (Cancrini et al. 2007). *Ae. albopictus* is an invasive species that has colonized every continent except

Antarctica in less than 40 years (Bonizzoni et al. 2014). The nature of this species, combined with a changing climate, may allow it to displace other mosquito species that have less medical importance. This potentially increases the spread of *D. immitis*, along with other pathogens, around the world (Juliano and Lounibos 2005). *D. immitis* has also been found to have a higher prevalence in urban areas than in rural or suburban areas, either because urban areas have a higher concentration of dogs or because *Ae. albopictus* uses containers for breeding (Gortazar et al. 1994, Marks and Bloomfield 1998).

While Oklahoma has a relatively high incidence rate of *D. immitis* infections in canine populations (Little et al. 2014), little is known regarding the principle vectors of this important parasite in the state. Historically, the main vectors for *D. immitis* in Oklahoma were considered to be *Culex erraticus* (Afolabi et al. 1989) and *Aedes trivittatus* (Afolabi et al. 1988). However, this research was only conducted in Stillwater, Oklahoma. In the 1990s, *Ae. albopictus* invaded Oklahoma and soon was found throughout most of the counties in the state (Noden et al. 2015). So, when an in-depth study was recently carried out to identify mosquito vectors of D. immitis in Oklahoma (Paras et al. 2014), *Ae. albopictus* was identified as the most important vector. However, the scope of the study continued to be only one county surrounding Stillwater, OK. There was a need for a wider study to focus on potential mosquito vectors of filarial worms in the state of Oklahoma. The aim of this study, then, was to test all of the mosquitoes collected during the summer of 2016 in 36 sites in six urban centers in Oklahoma and test them for the presence of mature filarial worms.

## Materials and Methods

Adult female mosquitoes were collected in six urban/peri-urban locations at each of seven different cities in Oklahoma between May 28, 2016 and September 20, 2016. Four cities (Lawton, Ardmore, Midwest City, Enid) each had 16 sampling events, Ardmore and Idabel had 15 sampling events, and Frederick had one sampling event. Sites were selected by proximity to urban centers, reported mosquito activity, location in relation to public centers such as parks, and reduced chances of trap disturbance. Oklahoma State University county extension agents aided in site selection by reporting mosquito problem areas in the cities.

At most sites, surveillance efforts utilized three types of mosquito traps: CDC Mini Light Traps (Bioquip, Rancho Dominguez, CA) with lights removed and baited with dry ice $CO_2$ released from modified insulated coolers, CDC Gravid Traps (John W. Hock Company, Gainesville, FL) baited with Bermuda grass-conditioned water, and BG-Sentinel® and BG-Sentinel 2® traps (Biogents, Regensburg, Germany) baited with BG-lure (Biogents) and octenol (Biogents). Samples obtained from Braggs, OK were collected with Light traps.

Mosquitoes were identified using keys from Darsie and Ward (2005). All mosquitoes were viewed at 4.25x magnification under a stereomicroscope and were identified to species. After identification, pools of species (*Ae. albopictus, Ae. sollicitans, Ae. triseriatus*, *Cx. pipiens,* and *Cx. tarsalis* were stored separately in 7 dram vials at -20°C. *Ae. albopictus* and *Cx. pipiens*) were selected due to having the highest rates of infection in a previous Oklahoma *D. immitis* survey (Paras et al. 2014). *Ae. triseriatus* was chosen due to having positive infections in this study as well. *Ae. sollicitans* was tested due to being shown as a competent vector in previous studies, though infection rates of this species have not been assessed previously in Oklahoma (Parker 2000). Other species were chosen due to high numbers found.

*Ae. albopictus, Ae. sollicitans, Ae. triseriatus*, *Cx. pipiens,* and *Cx. tarsalis* specimens were separated into pools of 10 or less individuals by type of trap used, site, and date of collection. These species were chosen because they were known vectors of *D. immitis* and represent the most numerous species of mosquitoes (almost half) collected in the survey. A total of 1,227 pools were processed from 7,446 mosquitoes: 548 pools (n= 3,836) of *Cx. pipiens,* 298 pools (n= 1,439) of *Ae. albopictus*, 140 pools (n= 878) of *Ae. sollicitans*, 130 pools (n= 828) of *Ae. triseriatus*, and 111 pools of *Cx. tarsalis* (n=465). The abdomens of the mosquitoes were removed in order to only test for mosquitoes infected with mature filarial worms which had migrated to the head and thoracic region. The heads and thoraces of the mosquitoes from each pool were added into a 2-ml sterile polypropylene Sarstedt microvials (Biospec, Bartlesville, OK) filled with 100 ml of DNAzol (Molecular Research Center, Inc., Cincinnati, OH). Samples were heated at 95°C for 15 minutes after which sterilized zirconia/silica beads (two large, six small; Biospec, Bartlesville, OK) (Biospec, Bartlesville, OK) were added and tubes were placed in a Mini-Beadbeater 16 (Biospec) for two minutes. After bead-beating, tubes were placed in a centrifuge at 12,000 rpm and run for one minute. The pooled supernatant was removed and placed into 1.7 ml tubes and frozen at -20°C until analysis.

Polymerase chain reaction (PCR) amplification was conducted in a separate laboratory than where the mosquito samples were processed in order to minimize DNA contamination. Screening of all samples was done using the screening primers DIDR-F1 (forward) and DIDR-R1 (reverse) that amplify a region of the internal transcribed spacer (ITS) of the ribosomal DNA as detailed in Rishniw et al. (2006). Each 25ul sample contained 12.5 ml GoTaq® Green Master Mix (Promega, Madison, WI), 10.5 ml DNAse/RNAse free $H_2O$ (Promega), 0.5 ml 5mM DIDR-F1, and 0.5 ml Mm DIDR-R1. Prior to placing the thermocycler, 1*u*l of mosquito supernatant from each pool was added to the reaction vials. For each PCR reaction, a positive control of 0.5 *u*l of *D. immitis* gDNA was added to a reaction vial as a as well as a water control as the negative control. Samples of *D. immitis* genomic DNA was provided by Dr. Rebecca Trout-Fryxell of University of Tennessee and Dr. Michael Reiskind of North Carolina State University. Verification of the positive samples included the use of the *D. immitis* specific primers (DI COI-FI/COI-R1) which amplify regions of the cytochrome oxidase subunit 1 (COI) gene as described by Rishniw et al. (2006). Primers specific for *Dirofilaria (Nochtiella) repens* and *Acanthocheilonema (Dipetalonema) dracunculoides* were not used because these species have not yet been detected in the US, and each positive DIDR F1/R1 sample was sequenced in an attempt to verify species.

Using the PCR reaction protocol outlined by Rishniw et al. (2006), the PCR procedure consisted of a denaturing step at 94˚C for 5 minutes and 32 cycles of denaturing (30s at 94˚C) annealing (30s at 60˚C) and extension (30s at 72˚C), a final extension (7 min at 72˚C) and a soak at 4˚C in a Bio-Rad C1000 Touch thermal cycler (Bio-Rad, Hercules, CA). All PCR products were analyzed by agarose gel electrophoresis in a 1x TAE buffer with 2% agarose gels stained with ethidium bromide. Gels were viewed with ultraviolet light. All results were photographed and printed for verification and documentation. All positive amplicons detected using the DIDR-F1/R1 primers were bidirectionally sequenced in the Oklahoma State University Core Facility. Resulting sequences were searched in the nucleotide BLAST database to determine the species of filarial detected in the specific mosquito pool.

**Results**

Of the 1,227 pools that were tested, seven (0.06%) samples tested positive. However, upon further testing using the DICOI-F1/R1 specific primers for *D. immitis*, only one sample tested positive for *D. immitis*. This result was confirmed by the sequence analysis using the BLAST database with a 94-96% sequence identity with known sequences of *D. immitis* (JX866681.1; JX481279.1; AF217800.1; KR676386.1). The positive sample was from a pool of nine *Cx. pipiens* captured in Idabel, OK with a CDC Gravid trap (Table 2).

One of the positive pools detected contained one *Ae. albopictus* taken from a BG Sentinel trap from a park/neighborhood interface in Ardmore, OK tested positive for the presence of nematode DNA. BLAST analysis revealed a 91% sequence identity with known sequences of *Brugia pahangi* (LK9666068.1; LKEU373638.1-55.1).

Additionally, four of the positive pools of *Ae. albopictus* from Ardmore and one pool of two *Ae. albopictus* from Midwest City tested positive for the presence of nematode DNA (Table 2). BLAST analysis of the positive amplicons revealed a 86-92% sequence identity with known sequences of *Mansonella ozzardi* (AF228559.1; AF228564.1; AF

254910.1; AF254908.1) Two of the samples were collected in CDC Mini Light traps, two from BG Sentinel traps, and one from a gravid trap (Table 1). None of the *Ae. sollicitans* or *Ae. triseriatus* samples tested positive for *D. immitis*.

**Discussion**

This is the first study that has identified DNA from several filarial species throughout the state of Oklahoma. In this study, only one of the 7 positive nematode samples had high sequence homology with known sequences of *D. immitis*. Interestingly, this sample was the only *Cx. pipiens* pool that was positive. It was collected at a location in the downtown area of Idabel, OK. Idabel, OK is located in McCurtain county, the most southeast Oklahoma county. McCurtain and its four surrounding counties are the only part of Oklahoma in the Southeast climate zone (Oklahoma Climatological Survey). McCurtain County has an average temperature of 17.2°C, compared to the state average of 15.5°C (Oklahoma Climatological Survey). Previous studies in Europe found a correlation between areas with higher temperatures and higher rates of infection of *D. immitis* than those with lower temperatures (Genchi et al. 2009). The higher temperature associated with McCurtain county may result in this area having a higher *D. immitis* infection rate. McCurtain county is also neighbor to two states, Arkansas and Texas. These states have *D. immitis* infection rates of 6.8% and 5.5% in dogs, respectively (Bowman et al. 2009). This is higher than the reported 2.1% infection rate across all of OK (Bowman et al. 2009). While McCurtain County is part of Oklahoma, it may be useful to compare this area with neighboring states that may have more counties that share a similar climate.

A pool of one of *Ae. albopictus* collected on September 17, 2016 in Ardmore, OK, had a high sequence match to *Brugia pahangi.* This species has not been found in North America, and is much more likely to be *Brugia beaveri*. *B. beaveri* is a parasitic nematode that was first described in raccoons in Louisiana (Ash and Little 1964). It has also been known to infect cats (Harbut and Orihel 1995) and there have been cases of human infection in the United States (Orihel and Eberhard 1998). There is little known about the natural history of this nematode, and *Ae. albopictus* has not been previously described as a vector. Most human infections of *Brugia* spp. are likely caused by *B. beaveri* or *B. lepori* (Orihel and Eberhard 1998). Though human pathogenicity of this nematode is not well known, *B. beaveri* was identified from a nocturnal blood smear taken from a boy in Oklahoma in 1984 in Oklahoma City (Leonard et al. 1984). What is known about *B. beaveri* is mostly from work done in Louisiana, south of Oklahoma and with a milder climate. The southern part of Oklahoma has an average rainfall of 97.87 centimeters, compared with the Oklahoma average of 85.75 cm. It is warmer, with an average temperature of 17.2°C, compared with the state average of 15.5°C (Oklahoma Climatological Survey). This climate may be more conducive to nematode infections than other parts of Oklahoma, if climate is a factor in transmission.

Finally, four pools *Ae. albopictus* from Ardmore and one pool of *Ae. albopictus* from Midwest City had a 90-96% identity with known sequences for *Mansonella ozzardi*, a human/primate filaria transmitted by blackflies and midges, with a range from southern Mexico to northern Argentina (Lima et al. 2016). Because the percent identity was low, it is unlikely these filiaria were *M. ozzardi* as this species has not been recorded in the United States. The farthest north it has been found has been the Yucatan Peninsula of

Mexico and the Caribbean islands of Trinidad, Haiti, and St. Vincent (Shelley and Coscarón 2001). It more likely to be *Mansonella llewellyni*, a filarid of raccoons in the United States. *Mansonella llewellyni* has little published data on its natural history and transmission. This is a parasite of raccooons and has been documented to infect raccoons at a rate of 14% in Florida (Telford and Forrester 1991). A study in Georgia found 65% of raccoons were infected (Pung et al. 1996). Little is known about the natural history and vector ecology of this species. It has been thought that *Ae. taeniorhynchus* is a vector, through *Ae. albopictus* has not been assessed for competence.

A study conducted in New Jersey, USA, found that *Ae. albopictus* fed exclusively on mammals, with over 90% of the blood meals derived from humans, dogs, and cats (Faraji et al. 2014). The author states that this mammalian host-feeding preference can make *Ae. albopictus* a good vector for many mammalian pathogens. Hawley (1988) found that *Ae. albopictus* will feed on a wide variety of hosts, and can even be induced to feed on snails. However, they greatly prefer mammalian prey, and humans are the most preferred (Hawley 1988). Also, 20% of *Ae. albopictus* in the field were found to have fed on multiple animals during each gonotrophic cycle (Hawley 1988). Delatte et al. (2010) states that *Ae. albopictus* is a generalist feeder on mammals, and this behavior may allow it to be a vector for a variety of pathogens. This can allow *Ae. albopictus* to pick up various parasitic nematodes, such as *M. llewellyni*, present in sylvatic mammal populations.

Midwest City is a metropolitan area located in Oklahoma County, Oklahoma. This county is in the central Great Plains area of Oklahoma, with an average temperature of

15.7°C, compared with the state average of 15.5°C (Oklahoma Climatological Survey). The temperature of Midwest City is very close to the state average, unlike the other locations in this study where nematodes were found. Midwest City, however, was found during a study to have similar mosquito diversity to Ardmore through canonical correspondence analysis (CCA) (Bradt, non-published data). This may be due to these two cities sharing similar longitudes, since Midwest City is directly north of Ardmore on the I-35 corridor. This may point to longitude being a factor in nematode prevalence, though this should be explored further in future studies.

The location of samples containing nematodes in Oklahoma is important from an ecological standpoint. Ardmore, OK had more PCR positive samples than all other cities surveyed combined. Ardmore is in Carter County, OK, which is separated from Texas by Oklahoma's Love County. Idabel, OK also is in McCurtain County, the most southeastern county in Oklahoma and is adjacent to Arkansas and Texas (Heck 1998). McCurtain County has annual rainfall of 132.13 cm, compared to the state average of 85.75 cm (Oklahoma Climatological Survey). The locations of nematode-infected mosquitoes collected may point towards milder climates being conducive to these parasites.

In this study, two PCR positive results were from CDC Gravid Traps. A study conducted in western Tennessee found that mosquitoes caught in CDC Gravid Traps had a significantly higher rate of *D. immitis* infection than those caught with CDC Miniature Light Traps (Trout Fryxell et al. 2014). CDC Gravid Traps are designed to attract and collect gravid female mosquitoes to increase the likelihood of capturing virus-positive mosquitoes, and may also be more attractive to females after blood feeding when parasites can be obtained and passed (John Hock, Gainseville, FL). Another study found that mosquitoes trapped in gravid traps had a higher rate of *Plasmodium* spp. infection than those caught in Encephalitis Virus Surveillance (EVS) traps, though caught a lower number of mosquito species (Carlson et al. 2015). It was speculated that this may be because gravid traps attract older mosquitoes that have fed and digested at least one blood meal. This leaves out some mosquito species that are not strongly attracted to gravid traps, such as *Cx. tarsalis* (Carlson et al. 2015). However, two nematode samples were from CDC Light traps, and three were from BG-Sentinel traps. This almost equal spread of trap types shows that in this study there is not strong correlation between trap type and nematode-positive samples.

The location of mosquitoes infected with *D. immitis* and other nematodes in this study can indicate prevalence of nematodes in urban areas as well as those with milder climates. The southeastern part of the state should be monitored in future studies as well as included in future mosquito-borne pathogen testing to see if there is a link between this area and higher rates of disease transmission. Such studies should employ multiple trap types.

**Importance of Higher Prevalence of Parasites in *Ae. albopictus* than other Species**

In this study, *Ae. albopictus* had a higher rate of infection of nematodes than other species. However, no *D. immitis* were found in *Ae. albopictus*. In a study in Taiwan, *Cx. pipiens* was found to have a higher infection rate with *D. immitis* than *Ae. albopictus* (Lai et al. 2001). Nayar and Knight 1999 found that not all *Ae. albopictus* individuals in southeastern Florida populations are able to transmit *D. immitis*. Some members of a population cannot survive being infected by this parasite, and die when the nematodes reach L3 within the mosquito. However, some members of the population are able to carry the parasite through its lifecycle, and serve as a vector for the pathogen. Subsequent generations from competent vectors in a population were able to carry more of the pathogen. This points to *D. immitis* competency being a heritable trait in *Ae. albopictus* populations (Nayar and Knight 1999). Another study conducted in Taiwan found that *Ae. albopictus* is a more efficient *D. immitis* vector than *Cx. pipiens* (Cheng-Hung et al. 2000). The higher nematode infection rate of *Ae. albopictus* as compared to *Cx. pipiens* in this study may point to populations of *Ae. albopictus* in Oklahoma being more efficient vectors of nematodes. Further studies should be conducted in order to compare the vector competence of *Ae. albopictus* in Oklahoma.

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**Table 1:** Number of pools tested positive for a parasitic nematode.

| Species | Number of individuals | Number of pools | Positive pools |
|---|---|---|---|
| *Aedes albopictus* | 1,439 | 298 | 6 |
| *Aedes sollicitans* | 878 | 140 | 0 |
| *Aedes triseriatus* | 828 | 130 | 0 |
| *Culex pipiens* | 3,836 | 548 | 1 |
| *Culex tarsalis* | 465 | 111 | 0 |
| Total | 7,446 | 1,227 | 7 |

**Table 2:** Date, location, trap type, and species testing positive for nematodes

| Date of collection | Mosquito species | Number of individuals | BLAST result | % Sequence Identity (Ascension #s) | Trap Type | City |
|---|---|---|---|---|---|---|
| | | | Filarial species | | | |
| 9-Jul-16 | *Culex pipiens* | 9 | *Dirofilaris immitis* | 94-96% (JX866681.1; KR676386.1) | Gravid | Idabel |
| 19-Jul-16 | *Aedes albopictus* | 1 | *Mansonella ozzardi* | 86-92% (AF228559.1; AF228564.1; AF254910.1; AF254908.1) | Light | Ardmore |
| 19-Jul-16 | *Aedes albopictus* | 10 | *Mansonella ozzardi* | | Sentinel | Ardmore |
| 20-Aug-16 | *Aedes albopictus* | 10 | *Mansonella ozzardi* | | Gravid | Ardmore |
| 3-Sep-16 | *Aedes albopictus* | 2 | *Mansonella ozzardi* | | Light | Ardmore |
| 13-Sep-16 | *Aedes albopictus* | 2 | *Mansonella ozzardi* | | Sentinel | Midwest City |
| 17-Sep-16 | *Aedes albopictus* | 1 | *Brugia pahangi* | 91% ((LK9666068.1; LKEU373638.1-55.1) | Sentinel | Ardmore |

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# CHAPTER V

## MOSQUITO MANAGEMENT IMPLICATIONS IN OKLAHOMA

ABSTRACT. There are 62 species of mosquito that have been found in Oklahoma, with some species known to be vectors of pathogens (Noden et al. 2015). Some of the pathogens transmitted by mosquitoes are not currently found in Oklahoma or the U.S., such as yellow fever virus. There are competent vectors of malaria, Yellow Fever, Dengue, Chikungunya, West Nile virus, and others that have been found in Oklahoma. Even if these diseases are not currently a problem, the presence of competent vectors shows the potential for future outbreaks. Because the surveys reported here were associated with urban centers, mosquito management should be a priority throughout Oklahoma. This study surveyed six cities between May and September during 2016. Although mosquito populations and communities are likely to change as a result of environmental fluctuations, the results from this study can be used to focus management by city.

**Idabel**

Idabel, OK had higher numbers of *Ae. albopictus* as compared to other cities sampled. This species originates from a subtropical climate in Southeast Asia (Hawley 1988). Idabel is located in McCurtain County, which has an average temperature of 17.2°C, compared with the state average of 15.5°C (Oklahoma Climatological Survey). It also has an annual rainfall of 132.13 cm, compared to the state average of 85.75 cm (Oklahoma Climatological Survey). The mild winters of this region and increased rainfall may maintain *Ae. albopictus*, which can transmit Chikungunya, Dengue, and other viruses (Guzzetta et al. 2016). *Ae. albopictus* prefers wetter areas than *Ae. aegypti*, and egg mortality is much greater for *Ae. albopictus* in dry periods than for *Ae. aegypti* (Juliano et al. 2002).

Idabel may also have larger numbers of containers used for breeding by *Aedes* mosquitoes, as 20.4% of the *Ae. albopictus* collected during this study were from one residential neighborhood in Idabel. This location had a large pile of tires behind a house, and the homeowner had called the local extension office to complain about the issue. During this study discarded tires were observed in various locations around the city. Elimination of these mosquito breeding sites should be prioritized to reduce mosquitoes in Idabel.

**Altus**

Altus is located in the Southwest climate region of Oklahoma in Jackson County, and has not been surveyed for mosquitoes previously (Oklahoma Climatological Survey). This study observed that Altus has large populations of saltmarsh mosquitoes, mainly *Aedes sollicitans*. *Ae. sollicitans* is commonly found in coastal areas of the United States, in salt marshes. This species was one of the first mosquitoes to have control measures carried against it, because of painful and persistent biting, making areas of Florida uninhabitable (Weissman 2016). This species may have also prevented coastal areas of New Jersey from developing into tourist areas prior to the advent of mosquito control (Headlee 1914). *Ae. sollicitans* is also a vector of Eastern Equine Encephalitis (EEE) and *Dirofilaria immitis*. A commonly used control method is "ditching", which creates ditches for marsh water runoff is used on much of the east coast of the United States to control this species (Kirby and Widjeskog 2013). The large populations of saltmarsh mosquitoes in Altus suggests the needs for management action. Altus also had the highest numbers of *Ae. aegypti* of any city in the survey area. This species is the only known vector of Zika virus, along with other viruses. In 2016 local transmissions of this virus have occurred in Florida (Likos et al. 2016). Management of *Ae. aegypti* has focused on reduction of breeding sources (Naranjo et al. 2014). In Singapore, reduction of *Ae. aegypti* breeding sites is enforced by law (Ooi et al. 2006). Management of *Ae. aegypti* should begin with a similar approach of reducing source sites, and on educating the public. Members of the public should be shown what mosquito breeding sites look like, and should be told how they can contribute by eliminating these sites at residences and businesses.

**Enid**

Enid's climate is very close to the average of Oklahoma, with an annual mean temperature of 15.6°C, compared to the state average of 15.5°C (Oklahoma Climatological Survey). It also averages 86.82 cm of precipitation, compared with the state average of 85.75 cm (Oklahoma Climatological Survey). These data and an average mosquito assemblage point to Enid being a good reference city for mosquitoes in central Oklahoma, and perhaps the historic mixed grass prairie region of the central U.S. Although there was a variety of mosquito species present in Enid, relatively large populations of *Ae. albopictus* were found. Enid may also be a good location to assess mosquito species survivability in Oklahoma, due to it being the northernmost area surveyed. Large populations of *Ae. albopictus* suggest that containers used by this species should be addressed in the city.

**Midwest City; Ardmore**

Midwest City and Ardmore, OK were found to have similar mosquito communities. Two species of concern that were found in these cities more than in other cities were *Culex nigripalpus* and *Aedes triseriatus*. *Ae. triseriatus* is the natural vector of La Crosse virus (LCV) (Bara et al. 2016; Bewick et al. 2016). LCV is the most common mosquito-transmitted disease native to the United States (Calisher 1994). It also is the leading cause of pediatric encephalitis in the U.S. (McJunkin et al. 2001). Approximately 100 cases of this disease are recorded in the United States every year (Leisnham and Juliano

2012). The high numbers of this species in these areas makes control of this vector very important.

*Cx. nigripalpus* is the most important disease vector in Florida (Day 2013). It is a competent vector of West Nile virus (WNV), St. Louis Encephalitis virus (SLE), and Eastern Equine Encephalitis virus (EEE) (Day et al. 2015). Its presence of large numbers in Ardmore makes this species important for public health and management should be considered. Adulticides are applied in Florida for control of this species, though there is little evidence to show this is effective. Cities in Oklahoma should monitor pools of water that form immediately after rainstorms, as these are the most favored breeding sites of this species (Day 2013).

Also, while no *Ae. aegypti* were found in Midwest City, during this survey, its close proximity to other cities which did have occurrences, like Lawton and Ardmore, make it an important place to watch for the emergence of this vector. This is especially true since it was found that Midwest City had similar mosquito species as Ardmore, which did have presence of *Ae. aegypti*.

**Lawton**

Lawton, OK, had larger numbers of *Anopheles* mosquitoes than other cities sampled. Three of these species, *An. quadrimaculatus*, *An. punctipennis*, and *An. pseudopunctipennis* are competent vectors of malaria (Malaria Atlas Project). Malaria is a global disease that kills over one million people every year (UNICEF). A 2013 study by the Centers for Disease Control found that there were 1,925 cases of malaria treated in the United States in 2011, which is the highest number since 1971 (World Health

Organization). Thus, the residents of Lawton should especially be made aware of malaria when travelling to countries where malaria is present. One objective should be to make sure residents are protected from malaria before they travel. If 90% of Americans are protected from malaria while they travel to malarious countries, the total number of malaria cases in the U.S. would drop to 192 (Dembele 2017). With the vectors for this parasite present in Lawton, possible transmissions should be closely monitored to prevent an outbreak.

**Mosquitoes Moving Across States Lines**

An important takeaway from the collection data is that *Ae. aegypti* appeared not only in an area that was never sampled before (Altus), but individuals were also found in areas of southern Oklahoma (Ardmore, Lawton) that had been sampled in previous surveillance efforts. *Ae. aegypti* was last recorded in an Oklahoma mosquito survey in 1940 (Rozeboom 1940). Since then, no Oklahoma surveys have collected *Ae. aegypti* in the state (Noden et al. 2015). Since *Ae. aegypti* was collected in these southern areas, it may be possible that this species moved north from Texas. Southern Texas is the only place besides Florida in the United States where *Ae. aegypti* populations are able to breed year round (Hahn et al. 2016). One management implication from this information is that areas along the Red River border of Oklahoma-Texas should be monitored for *Ae. aegypti* populations, as well as for other tropical mosquito species that may move northward during warm weather. Since this survey did not look at all cities along southern Oklahoma, it would be reasonable to assume that many other cities along the border with Texas may also harbor *Ae. aegypti* populations. Cities in southern Oklahoma should consider monitoring for new and invasive mosquito species.

## Oviposition Sites

Large mosquito populations in many Oklahoma cities are likely because of availability of oviposition sites being created inadvertently by humans. In Altus, Frederick, and Idabel, large tire piles were frequently encountered. *Ae. aegypti* and *Ae. albopictus* are both strongly associated with tire piles, and these sites can be sources of mosquitoes for an entire surrounding community (Lounibos et al. 2016). *Ae. aegypti* were collected near tire piles in Altus and Frederick, but not in Idabel. However, in Idabel one residence with a tire pile behind it produced 41% of all the *Ae. albopictus* captured in this study. A study in Mississipi found that around 70% of mosquitoes sampled from tires were *Ae. albopictus* (Yee et al. 2015). This study also found that the environments where tires are located influence the species found, though *Ae. albopictus* were found in a variety of tire conditions (Yee et al. 2015). City managers need to be aware of tire piles as mosquito habitats.

Another source of mosquito breeding observed during this study were areas of poor drainage. Multiple locations in Ardmore exhibited poor drainage and produced large numbers of mosquitoes. One of these was directly behind the downtown area of the city, and *Ae. aegypti* were collected there. Another drainage area at a local park was associated with *Ae. aegypti*. A wooded area behind a high-income neighborhood had fluctuating levels of water, and were associated with high numbers of *Ae. triseriatus* (41% of total) and *Cx. nigripalpus* (34% of total). A study conducted in Argentina found that areas of poor drainage produced large numbers of mosquitoes (Quiroga et al. 2013).

Those areas that received water from leaking pipes produced more mosquitoes than those that were naturally filled (Quiroga et al. 2013). This study suggests that drainage areas in urban areas that have leaking pipes as a water source being a potential problem from a mosquito standpoint. This mosquito management issue should be addressed by cities with this problem can support mosquito populations.

**Pathogens in Oklahoma**

While 1,227 pools of ≤10 mosquitoes comprising *Ae. albopictus*, *Cx. pipiens*, *Ae. triseriatus*, and *Ae. sollicitans* were tested, only one sample was positive for *Dirofilaria immitis*, the causative agent for dog heartworm. This sample was found in a mosquito collected in Idabel. While this was the only city with an example of *D. immitis* found, it is difficult to draw conclusions from a single sample. However, no other study in Oklahoma has compared *D. immitis* infection rates in mosquitoes across different areas of Oklahoma. The detection of this pathogen in Idabel can imply a higher presence of dog heartworm in Southeastern Oklahoma. Alternatively, the low amounts of *D. immitis* found in this study may point to pet owners implementing effective *D. immitis* treatment of domestic dogs.

Other parasitic nematodes were also found during this survey. One sample had a high sequence match to *Brugia pahangi*, which is likely *B. beaveri*. This nematode has been known to infect humans and was observed in an Oklahoma child in 1984 (Orihel and Eberhard 1998; Leonard et al. 1984). However, it is not noted as a significant public health risk. Five samples showed a high sequence homology to *Mansonella ozzardi*, though they are likely *M. llewellyni*, which is a parasite of raccoons with an unknown effect on humans (Telford and Forrester 1991). Additional testing and awareness of parasitic nematodes is warranted based on this study.

**Importance of Using Multiple Trap Types**

An important outcome from this study was the importance of using multiple mosquito trap types to assess mosquito species diversity. All mosquito trap types used in this 2016 survey caught significantly different species of mosquitoes. Because of this observation, using only one trap type for a mosquito survey may insufficiently assess mosquito populations in an area. The important vector *Ae. aegypti* was found in Lawton from one specimen captured in a CDC Gravid trap. Interestingly, gravid traps are made to attract *Culex* mosquitoes, while BG-Sentinel traps are designed to attract *Aedes* (Lee et al. 2016; Li et al. 2016).

**Traps for Mosquito Management**

While mosquito traps are used for surveillance of mosquitoes, their use as a control measure has not been extensively explored. Mosquito control in the United States relies on insecticide use (Rose 2001). Environmental implications and concerns generated by this management technique have caused a decrease in insecticides approved for mosquito control (Rose 2001). There are concerns that mosquito trapping may not be successful due to a much higher potential for population increase.

There have been successful examples of mosquito control using traps. A study in 1999 in Salt Lake City, Utah, found that homeowners reported relief from the pest *Ochlerotatus sierrensis* after an abatement program using MM-Pro traps (Woodstream Corp., Lititz, PA) was implemented (Hougaard and Dickson 1999). A study conducted on several small islands off the coast of Cedar Key, Florida, involved using traps for management. From May through October these islands were unsuitable for human visitation due to aggressive feeding by *Ae. taeniorhynchus*. Baited MM-Pro traps significantly reduced biting activity after 2 weeks of trapping. After trapping, repellent applications to exposed skin were not needed (Kline et al. 2012). Another study conducted in urban areas of Puerto Rico assessed the use of the CDC autocidal gravid ovitrap (AGO) (CDC, Atlanta, GA) as a method of control for *Ae. aegypti*. The traps were compared with BG Sentinel traps (Biogents, Regensburg, Germany). BG Sentinel traps captured more mosquitoes per unit of time than AGO traps, but also caught more males. Both of these traps resulted in a significant decreases in *Ae. aegypti* presence in the areas tested (Barrera et al. 2014). Thus, it appears that various mosquito traps can be a viable method to reduce mosquito populations, and could become part of an integrated pest management program.

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APPENDIX I

# COLLECTION OF LARVAL AND PUPAL MOSQUITOES

On 18 July 2016 larval and pupal mosquitoes were collected from six tires at a site in Altus, OK. A standard meat baster was used to remove larvae and pupae from the tires. Mosquitoes were reared in a growth chamber at a temperature of 25°C and a 14:10 light/dark period. Once adults eclosed, they were frozen at -20°C and identified to species using keys from "Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico" (Darsie and Ward 2005).

## Tire Samples

A total of 197 mosquitoes were collected from the six tires sampled. Eight species were collected (Table 1). The most common species was *Cx. coronator.* The same amount of *Cx. coronator* were captured from the tires (n=87) than from the four-month sampling study (n=87). *Cx. coronator* is an important invasive species that has been recently reported outside of its known range of New Mexico, Texas, and Arizona. It has been found in Louisiana, Georgia, Mississippi, Florida, and Alabama (Connelly et al. 2016). They have been documented as a tire-dwelling species. Only one specimen was collected previously in Oklahoma, in a 2003 survey (Noden et al. 2015). Only two adult specimens were collected at the site that contained the tires sampled. There were also 71 *Cx. restuans* found in the tires, when only 19 were collected through trapping at this site. Ten *Cx. tarsalis* were collected from the tires compared to 15 from traps during the four-month study. These difference in tire collection compared to trap collections may point to the importance of assessing the differences between baited trap collection and actual mosquito presence in an area.  Future studies should investigate if these discrepancies are common when surveying mosquito populations.

**Table 12.** Numbers and species of mosquitoes collected from tires in Altus, OK on 18 July 2016.

| Tire | *Ae. albopictus* | *Cx. coronator* | *Cx. tarsalis* | *Ae. aegypti* | *Cx. pipiens* | *Cx. restuans* | *Ae. epactius* | *An. quadrimaculatus* | Total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 37 |
| 2 | 6 | 1 | 0 | 1 | 0 | 15 | 0 | 0 | 23 |
| 3 | 7 | 42 | 0 | 1 | 3 | 0 | 0 | 0 | 53 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 1 | 15 | 0 | 0 | 56 | 0 | 0 | 72 |
| 6 | 1 | 7 | 0 | 1 | 0 | 0 | 2 | 1 | 11 |
| Total | 15 | 87 | 15 | 3 | 3 | 71 | 2 | 1 | 197 |

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