EPIZOOTIOLOGY OF DOG HEARTWORM,

DIROFILARIA IMMITIS, IN OKLAHOMA

By

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CHAPTER I

INTRODUCTION

Dirofilaria immitis is a mosquito-borne nematode that causes a serious, fatal disease in dogs and cats. Although this disease can be prevented with the use of anthelminthic drugs, many dogs and cats remain at risk because they are not given adequate preventative medicine (Bowman 2009). This disease is important to pet owners due to the devastating effects on untreated animals, and much of the research conducted has looked at infected definitive hosts only, with a focus on prevention in companion animals. Consequently, our understanding of the ecology and epizootiology of *D. immitis* is limited. It is particularly important to learn what species of mosquitoes are transmitting heartworm in any given region to focus local vector control efforts. Previous heartworm vector studies conducted in Payne County were conducted in the 1980s and mosquito species composition has changed in that time, so different species may be important now than in 25 years ago (Afolabi 1989). Furthermore, there is also a lack of knowledge regarding the prevalence rate of heartworm in coyotes in Oklahoma, which may serve as reservoir hosts. There is great variation between similar coyote prevalence studies throughout the country, with higher rates in the east than the west, so documenting the prevalence in Oklahoma increases knowledge about the importance of wild canids in the disease cycle and the effects of geography. Oklahoma is an ecologically diverse state with eleven recognized level three ecoregions spanning the state (Environmental Protection Agency 2000). A study in Oklahoma may show relationships between ecotype and definitive host infection. Furthermore, dog heartworm can serve as an excellent model system of vector-borne disease, providing a framework to test basic questions about the relationship between landscape and social factors and disease risk.

There are three main objectives of this study. The first is to determine the prevalence of *Dirofilaria immitis* in coyotes throughout Oklahoma. The second is to evaluate the relationship between landscape and socoeconomic factors and heartworm positive mosquitoes. The third objective is to incriminate the most important mosquito species transmitting *D. immitis* in Payne County, Oklahoma, with a specific focus on evaluating the relative importance of the invasive Asian tiger mosquito, *Aedes albopictus* in heartworm transmission.

The hypothesis for the first objective is that coyotes in Oklahoma have a prevalence rate of *D. immitis* intermediate between studies from the eastern and western United States. It is predicted that coyotes collected from ecoregions with more precipitation will have higher prevalence rates. The second hypothesis is that certain landscape and social factors exist that cause differences in mosquito infection rates of *D. immitis.* From this hypothesis, it is predicted that landscape factors will predict the likelihood of finding heartworm positive vectors. Although previous research has been conducted to determine important vectors of *D. immitis* in Oklahoma in the past (Alfolabi et al. 1989), changes in vector community since the mid-1980s suggest that different species may now be responsible for dog heartworm transmission. Therefore, it is hypothesized that the invasive *Ae. albopictus* is an important vector of heartworm in Payne County. It is predicted that *Ae. albopictus* will have high infection rates as has been shown throughout its native and invasive range (Cancrini et al. 2003, Lee et al. 2007, Licitra et al. 2010).

The format of this thesis follows the journal manuscript format. Following this brief introduction, Chapter II is a literature review of the information surrounding *D. immitis*, coyotes, and mosquito vectors. Chapters III and IV are stand-alone manuscripts that will be submitted for publication. Chapter III details the prevalence rate of *D. immitis* in coyotes in Oklahoma and North Texas.

Chapter IV explains the vector incrimination study in urban and rural Payne County, Oklahoma. Chapter V is a brief summary and conclusions chapter. The literature review and each stand-alone manuscript have appropriate literature cited sections associated with them.

CHAPTER II

LITERATURE REVIEW

Introduction

The dog heartworm disease cycle includes *D. immitis,* the pathogen, mosquito vectors, and domestic and wild canines as the definitive hosts of the disease. It is necessary to examine each component for complete knowledge of the disease. Important areas of the disease cycle include the pathology, diagnosis, treatment, and prevention of dog heartworm, epizootiology of dog heartworm in domestic and wild canids worldwide, and mosquito biology and transmission.

Pathology, Diagnosis, Treatment and Prevention

Dog heartworm is a chronic, ultimately fatal infection of companion dogs and cats, caused by *Dirofilaria immitis* Leidy, and spread by the bite of over 60 species of mosquitoes (Ludlam et al. 1970). *Dirofilaria immitis* is a nematode, filarid parasite in which the adults primarily infect the pulmonary artery and right ventricle of wild and domestic canines. The female worms give birth to microfilariae that circulate in the bloodstream of the definitive (dog) host. The intermediate host, a mosquito, ingests the microfilariae when she takes a blood meal.

The microfilarie develop to third stage larvae in the mosquito's malpighian tubules over a period of 10-15 days (Foster and Walker 2009). The infective juvenile worms migrate to the head capsule of the mosquito and enter a definitive host when the mosquito feeds again. The larva stays near the bite wound for several days before molting. Over the next two to three months the larvae molts again and becomes an adult and migrates to the pulmonary artery. After another three to four months, the infection becomes patent and detectable, and following mating the adult females begin producing microfilariae.

Cats are also susceptible to infection with *D. immitis*, but are not competent hosts. A few *D. immitis* larvae can mature to adulthood in the aberrant cat host, but they are rarely able to reproduce and do not produce sufficient microfilaremia to infect mosquitoes (Bowman 2009). Despite the low parasite load, infected cats can develop heartworm-associated respiratory disease, a serious condition caused by the migration of larvae through the lungs (Blagburn and Dillon 2007). Migration of the larvae throughout the cat can also result in sudden death (Bowman 2009). Infection in cats, as in dogs, is ultimately fatal.

Depending on parasite load, infected canines can experience four classes of disease. Precise numbers of worms that cause disease in dogs varies with the size and overall health of the animals. Smaller dogs and those that have other health problems are less tolerant of infection than larger dogs, and because of their smaller cardiovascular system, small dogs become symptomatic with lower numbers of *D. immitis.* Class one disease results in a subclinical infection. Class two is characterized by the onset of signs. The signs are generally mild and consist of a chronic cough, dyspnea, and reduced exercise tolerance. In class three disease, the dog shows more severe signs, including syncope, hemoptyses, congestive heart failure, and ascites. Class four disease is the acute onset of heartworm disease and is also known as vena cava syndrome. If a dog is at this level of illness, surgery is the only option to remove the worms via the jugular vein. Without surgery the dog will die within 24 to 72 hours (Bowman and Atkins 2009).

Although currently prevented with a prophylactic ivermectin, milbemycin oxime, selamectin, and moxidectin regime, once an infection occurs, treatment is much more difficult and expensive (Bowman 2009). The cost of monthly prophylaxis varies for the type of drug, the company, and the size of the dog. For example a medium-sized dog on monthly preventatives purchased from a national drug dispenser will cost approximately $67 per year (1800PETMEDS.com 2010). In spite of the low cost, not all dog owners and even fewer cat owners comply with recommend prevention regimes, and compliance among indoor pet owners is particularly low, based on the false assumption that the mosquito vectors do not enter houses. The emergence of resistance to ivermectin in other filarids raises the possibility of resistance in *D. immitis*, which would make vector control an important component in preventing transmission (Prichard 2005).

Treatment options vary depending on the severity and duration of the infection. For dogs with a mild to moderate clinical signs, two injections of melarsomine dihydrochloride can be given 24 hours apart. Moderate to severe infections require two melarsomine dihydrochloride injections given one month apart to increase efficacy of the treatment by allowing any immature worms to mature before another application of adulticide and to allow the infected animal to clear the dead worms without shocking their system (Bowman and Atkins 2009). An animal suffering from acute disease, vena cava syndrome, requires surgery for successful treatment (Bowman 2009). During all treatment the cardiopulmonary system is heavily stressed, so exercise must be restricted to prevent death (Bowman 2009).

Occult infections are those in which the dog is infected with heartworm, but the infection is not detectable (Bowman 2009). Several reasons for having an occult infection exist. The dog can have a single sex infection, in which only males or only females are present, resulting in no microfilariae production. Early infections reduce detectability before maturation of the worms and production of microfilarie. Cats have an especially strong immune response to the *D. immitis* worms, which typically results in maturation of a few adult worms, but no circulating microfilariae. Inconsistent use of avermectins by pet-owners can kill the circulating microfilariae, but not the adult worms. This irregular drug use likely accounts for the majority of occult infection (S. Little personal communication). Occult infections are dangerous because the dog will not receive appropriate care and thus may contribute to the further transmission of disease.

There are three tests which can be used to determine if an animal is heartworm positive. In the simplest test, the animal is killed and the heart dissected. The animal is positive if adult worms are observed in the heart tissue. A second, non-lethal method is the modified Knott's test which looks for circulating microfilariae in the bloodstream (Zajac and Bellows 2006). The veterinarian draws one ml of blood and lyses the red blood cells with a 1:10 dilution with formalin. The microfilariae are sediment stained with methylene blue. The number of microfilariae are counted. The final, most common test is a SNAP test which detects antigen given off by the female reproductive tract. This test is commercially available. Three drops of whole blood or serum are added to four drops of the provided conjugate. This solution is mixed by inversion and poured onto the test. When the liquid is absorbed across the test, the activator button must be fully compressed. The veterinarian must wait eight minutes for test results (IDEXX Laboratories, Inc.).

Problems exist for both lethal and non-lethal tests. Of the two non-lethal tests, the SNAP test is generally more reliable and accurate than the modified Knott's test. The modified Knott's test is now out-of-date for determining infection for several reasons. Occult infections in which there are no circulating microfilariae in the bloodstream could lead to false negatives. Additionally, the process of infection to production of microfilariae takes approximately 6-9 months. It only takes 4-5 months for nematodes to mature in the host, so using a SNAP test can detect the infection sooner than a Knott's test, shortening the window of false negative results (Bowman 2009). Because the Knott's test only uses 1 ml of blood, it is has low sensitivity compared to the SNAP test which can detect the antigen from the reproductive tract of as few as one to three adult female worms. The antigen test detects a female reproductive tract antigen, so the SNAP test will only work once the females have matured. The antigen test does nothing to shorten the pre-patency period. If an animal is killed or has died, cardiac dissection can be performed at necropsy to visually inspect the pulmonary artery for adult worms. The problem with this method is that due to human error and the degree of decay of the carcass, it is possible to miss the worms.

Biology and Natural History of Coyotes, *Canis latrans*

Coyotes have been shown to be infected with heartworm throughout the United States (Wixsom et al. 1991, Custer and Pence 1981, Foryet 2008, Sacks et al. 2004, Foster et al. 2003), however no studies have been conducted to determine the prevalence of heartworm in coyotes in the southern plains region. Learning about their role in the southern plains is important in the understanding of heartworm transmission and the role that coyotes play in the heartworm disease cycle in this ecosystem and location. Coyotes have been able to thrive in human altered landscapes because higher predators, such as wolves and bears, have been eliminated and because anthropogenic food is readily available for scavenging (Andelt 1985).

Coyotes are omnivores, feeding on fruits, small vertebrates, and larger mammals (Andelt 1985). For feeding on fruits, grasses, insects, and small vertebrates such as rodents and birds, coyotes are able to catch food on their own without assistance from other coyotes (Andelt 1985). The amount of cooperation between coyotes increases as the size of the prey increases. When hunting larger prey such as rabbits, raccoons, opossums, armadillos, skunks, and deer fawn, pairs of coyotes may work together to capture the prey and share the food (Andelt 1985). For even larger prey items, such as deer, cattle, javelinas, and feral hogs, cooperation becomes obligatory to capture the prey and, as in other cooperative hunting behaviors, the coyotes share the food (Andelt 1985). Diet composition is dependent on the time of year due to food availability. In a study of coyote diet by Andelt (1985), fruit made up the majority, 65%, of the diet in the summer months and only 1% during the winter months. Mammals were determined to account for 87% of the winter diet and 28% of the summer diet. Predictably, during fawning season, white-tailed deer are a common prey item. Similarly, during the summer insects were important in the diet and small mammals such as mice and rats were important prey items in the winter. Juveniles are able to hunt small rodents such as voles and mice as well as insects, while only adults hunt larger mammals including ground squirrels and rabbits (Wells and Beckoff 1982). Cattle, probably scavenged carcasses, were also part of the coyote diet during the winter (Andelt 1985). Studies about diet composition are based on scat analysis, so it is impossible to determine what proportion of large prey animals were killed or scavenged (Andelt 1985). In a study that observed the predation, all but one ungulate was eaten as carrion (Wells and Beckoff 1982).

In Oklahoma, coyotes are able to breed beginning in December, earlier than in northern states, and will continue to breed until March (Dunbar 1973). They are long day monoestrus breeders, meaning females go into heat once per year in late winter and early spring as day length is increasing (Knowlton 1972). The females come into heat in January-March. The gestation period is 60-63 days and the pups are born in March-May (Knowlton 1972). Average litter size in Texas was 6-7 pups (Andelt 1985), and is probably similar in Oklahoma. Both male and female parents are responsible for raising the pups (Andelt 1985). Males are sexually mature at approximately one year old, while females two years and older have higher fecundity than yearlings (Sacks 2005).

Coyotes live alone, in pairs, or in groups of 3-7 related individuals. Individuals have a home range of 4.3 to 4.7 sq km (Andelt 1985). The average group size was 1.4 to 1.8 individuals because group size changed depending on the time of year. The group size was larger in the winter during the breeding and gestational time period than in the spring and summer during whelping and post-nursing stages (Andelt 1985). Young males tend to emigrate and find a new home more often than young females (Andelt 1985). They emigrated an average of 13 km away from their last known home range (Andelt 1985).

Coyotes have been implicated as major livestock pests and as a result have been subject to predator control (Beckoff 1978). This is ineffective, because coyotes exhibit compensatory natality. Under high predator control, they will produce more offspring to compensate for the increased mortality (Knowlton 1972). When coyotes are under high mortality stress from hunters, the coyotes that are not killed are able to survive winter more readily than when there is a high density of coyotes in the area (Beckoff 1978, Wagner 1975).

Coyotes have been able to thrive in urban areas of the United States (Gehrt 2007). Survival rates in major urban centers are high: in Chicago the survival rate is 0.62, Tucson, Arizona 0.72, and Los Angeles, California 0.74 (S. Gehrt unpublished data, Grinder and Krausman 2001, Riley et al. 2003). An estimate of the coyote survival rate in a rural area outside of Albany, New York is 0.20 (Gehrt 2007). The higher survival rates recorded in urban areas than in some rural ones could be caused by a lack of hunting in urban areas (Gehrt 2007). The home range of urban coyotes is 7.3 sq km, which is larger than reported rural home ranges (Gehrt 2007). In highly urbanized areas the home range increases and coyotes are more likely to live solitary lives, presumably to compensate for lack of resources (Gehrt 2007). The diet of urban coyotes, in order of importance, consists of rodents, rabbits, human-related items, and domestic cats (MacCracken 1982 and Morey et al. 2007).

Epizootiology of Dog Heartworm in Domestic and Wild Canids

Nationally, dog heartworm has been increasing its range in the last 30 years, in spite of effective preventative treatment (Weinman and Garcia 1970, Pennington et al. 1970, Kocan 1976). Heartworm infections in domestic dogs in California were not diagnosed until the 1970s (Weinmann and Garcia 1974). A current, nationwide survey reveals that the worm has been reported in domestic dogs throughout the continental United States (Bowman et al. 2009). *Dirofilaria immitis* spread into Oklahoma between 1969 and 1974 (Pennington et al. 1970 Kocan 1976). A survey conducted in the summer of 1969 of 100 shelter dogs from Stillwater, Guthrie, Edmond, Enid, and Ponca City revealed an infection rate of 0% (Pennington et al. 1970). A survey conducted five years later from 1974-1975 showed the infection rate of dogs being treated at Oklahoma State University veterinary hospital was 4.5% with circulating microfilariae and 7.3% presenting adult heartworms at necropsy, suggesting an invasion of Oklahoma with *D. immitis* in the early 1970s (Kocan 1976). Owners of some infected dogs reported that the dogs had never been out of the state indicating that the filarid parasite had become enzootic in Oklahoma (Kocan 1976). The only recent data available indicated a 2.1% infection rate in Oklahoma dogs (Bowman et al. 2009).

Current research shows that at 3.9%, the southeastern states had the highest prevalence rate in the country, while Oklahoma had a prevalence of 2.1% infection (Bowman et al. 2009). Lincoln county reported the highest infection rate in Oklahoma , > 6.1%, however, many counties had fewer than ten test results, so information from those counties were not included in the analysis (Bowman et al. 2009). If the number of individual test results reported was greater than ten, but still low, the infection rate could appear inflated (e.g., one positive test out of ten shows an infection rate of 10%). This type of large scale survey provides insight into general trends of disease over a large spatial scale like the continental United States, but does not explain variation at the more local scale that dictates risk of infection for each pet. Smaller scale vector studies looking at habitat and host availability can answer detailed questions about risk factors, including urban versus rural landscapes and host density, associated with *D. immitis* infection.

In addition to the spread of dog heartworm in the United States, it has been spreading in other regions of the world. Historically in Italy, dog heartworm was only found in the northern regions, but in recent years has spread into southern areas of the country (Otranto 2009). Reasons for this spread remain obscure, but may be associated with changes in distribution of mosquito vectors. Little is known about how these changes might affect spread of dog heartworm in the United States.

Although domestic dogs tend to be the focus *D. immitis* research, wild canids are potential reservoirs of the disease (Weinman and Garcia 1980). Prevalence in wild canids seems dependent upon location, with the prevalence of heartworm in coyotes lower in Western states than Eastern states. Oklahoma is an ideal location to study the dog heartworm in coyotes because it is ecologically diverse. There are eleven level three ecoregions spanning the state (Environmental Protection Agency 2000). Some researchers in other parts of the country considered all age classes of coyotes together when reporting infection rate data. Of 24 coyotes collected along the Gulf Coast including Texas and Louisiana, 17, or 71 % of the coyotes were infected (Custer and Pence 1981). In Washington state, researchers used cardiac dissection as a detection technique and found none of the 556 coyotes tested were infected (Foryet 2008). Sacks et al. (2004) collected coyotes at the California county level and found a wide range of prevalence rates, 0-25% . Forty-three percent of coyotes collected in Florida were found to be heartworm positive (Foster et al. 2003), while coyotes collected from rural areas of Illinois revealed a 16% prevalence rate (Nelson et al. 2003). As in domestic dogs, heartworm is probably spreading in wild canids. For example, into the early 1990s, heartworm infections in wild canids were low, but increased to 91% by 1996 in a small sample of 23 coyotes collected in California (Sacks 1998).

Studies have also examined the effects of age and geographic location on percentage of coyotes infected with heartworm. A study in Missouri looked at infection rates between age classes. Age class is determined by width of the pulp cavity of the lower canine tooth (Kuehn and Berg 1981). Predictably, coyotes that are older, up to 3.5 years of age, were significantly more likely to be infected (Wixsom et al. 1991). An infection, once established, is not self-limiting, as older coyotes have had more time to be exposed to mosquitoes and *D. immitis.* However, coyotes that were older than 5 years began to again show a decrease in infection rate. Wixsom et al. (1991) hypothesized that older coyotes that may be weakened from other health problems were more likely to have died from infection and therefore there was a survival bias in the sampled coyote population (Wixsom et al. 1991). In Missouri, coyotes less than six months old had an 8.7 % infection rate while those over 3.5 years had 40.4 % infection rate.

Although domestic dogs and cats suffer severe, deadly disease associated with *D. immitis* infection, observations of carcasses did not reveal major differences in apparent health between infected and uninfected coyotes (Nelson et al. 2003). In Illinois, heartworm-infected yearling female coyotes were significantly less likely to have reproduced than those that were uninfected (Nelson et al. 2003). This difference was not maintained throughout the other age classes. Older female infected and uninfected coyotes were equally likely to have reproduced (Nelson et al. 2003).

Male and female wild canines have not been shown to have significant differences in infection rates (Nelson et al. 2003). In terms of coyotes, 1.07 females were infected to every 1 male (Nelson et al. 2003). In Texas and Louisiana, when looking at wild canines together, 85 % of males and 76 % of females had *D. immitis* worms in their pulmonary artery (Custer and Pence 1981).

Other wild canid species, including red wolves, *Canis rufus gregori,* coyote x red wolf hybrids, and red foxes, *Vulpes vulpes*, can also be infected with *D. immitis.* Coyote x red wolf hybrids had an 83 % infection rate for 46 specimens collected in Texas and Louisiana (Custer and Pence 1981). Low numbers of red wolves (*n* = 8) were collected, but all of them (100%) were infected with heartworm (Custer and Pence 1981). Due to the low number of specimens collected, it is impossible to extrapolate an accurate infection rate for the population, however, this is valuable in that it is known that this species is able to be infected. Red foxes were not found to have such high infection rates. Of 85 red foxes collected in Missouri, only 6 % were infected with *D. immitis* (Wixsom et al. 1991).

In spite of relatively high infection rates and the diversity of wild canids that can be infected with *D. immitis,* some researchers have hypothesized that wild canines are not an important reservoir of disease for the domestic dog population; rather, the infections in domestic animal populations are the cause of the infections in the wild canine population (Otto 1969). This hypothesis is supported by research conducted in Melbourne, Australia, to test the effects of location (urban or rural) on *D. immitis* infection in red fox (Marks and Bloomfield 1998). The infection rate in Melbourne was 6.4% of 93 foxes tested, compared to none of the 19 foxes tested from rural surrounding areas (Marks and Bloomfield 1998). This phenomenon was also observed in Spain in which foxes collected from riparian areas, between high human populations and rural areas, had higher infection rates than those collected in more secluded areas (Gortazar et al. 1994). From these data, it has been hypothesized that higher concentrations of domestic dogs in urban centers drives the elevated wild canid infection rate in cities. In rural areas where there is a lower density of domestic dogs, the infection rate in wild canids is also decreased.

Mosquito Biology and Transmission of *D. immitis*

There have been numerous studies in the United States over the last forty years that have demonstrated a variety of potential vectors, including mosquitoes in the genera *Anopheles*, *Psorophora, Culex* and *Aedes* (Eldridge and Edman 2000). Researchers conducted a study of the presence of L3 larvae in mosquitoes collected from Gainesville, Florida, Bartow, Florida, and Baton Rouge, Louisiana (Watts et al. 2001). In Gainesville, Florida *Ae. canadensis, Ae. vexans, An. crucians, Ae. infirmatus, Cx. nigripalpus,* and *Ps. ferox* were all found to be positive for *D. immitis. Anopheles crucians* had the highest rates of infection with 0.2% (Watts et al. 2001). In

Bartow, Florida only *Ae. vexans* were positive with heartworm at a rate of 2% (Watts et al. 2001). The researchers found positive pools of *Ae. vexans, An. quadrimaculatus,* and *Ps. columbiae* in Baton Rouge, Louisiana (Watts et al. 2001). In more recent studies in Georgia, researchers found three species of mosquitoes to be heartworm positive including, *Ae. albopictus, An. punctipennis*, and *An. crucians* (Licitra et al. 2010).

The only work on vector incrimination in Oklahoma was conducted nearly thirty years ago. This vector study work incriminated *Aedes trivatattus* (Coquillet) and *Culex erraticus* (Dyar and Knab) as the important *D. immitis* vectors in Oklahoma based on mosquito feeding habits, numbers of mosquitoes, and ability to transmit heartworm (Afolabi et al. 1988, 1989). However, this study was conducted at a single site in Payne County over a short period of time, using a dog infected with *D. immitis* as bait, thus preventing any examination of underlying landscape or socioeconomic factors that may contribute to dog heartworm transmission. In addition, the vector community in Oklahoma has changed since this study in the late 1980s.

A dramatic change in the vector community of Oklahoma has been the invasion by the Asian tiger mosquito, *Aedes albopictus* Skuse (Hawley 1988). This mosquito is a container-breeding, synanthropic mosquito originally found in East Asia (Hawley, 1988). It is an important vector of numerous human pathogens, including dengue virus, chikungunya virus, and the filarid nematode that causes human filariasis, *Burgia malayi* (Gratz, 2004). Throughout its native home range, *Ae. albopictus* has been shown to be a vector of heartworm. Although found to have lower infection rates than *Cx. quinquefasciatus* in Taiwan, *Ae. albopictus* is an important vector on this island nation (Lai et al. 2001). *Aedes. albopictus* is known to be an important vector of *D. immitis* larvae in Singapore, Japan, China and Korea (Chellappah and Chellappah 1968, Konishi 1989, Lai et al. 2000, Lee et al. 2007).

In laboratory experiments, *Ae. albopictus* maintained an average of 20.7 *D. immitis* larvae and up to 51 larvae for up to twelve days post-infection, and 99.4% contained at least one maturing larvae, indicating that *Ae. albopictus* is a competent vector of dog heartworm (Kartman 1953). The potential for the global invasion of this mosquito to contribute to the spread of disease has been suggested (Juliano and Lounibos, 2005), although empirical evidence has been limited. In Italy, which *Ae. albopictus* invaded in the 1990s, this species has been shown to be an effective and important vector of heartworm (Cancrini et al 2003). Furthermore, the spread of *D. immitis* from northern into southern areas of Italy may have been driven by the concomitant invasion of these parts of Italy by *Ae. albopictus* (Otranto et al. 2009)

Urbanization and Mosquito-borne Epizootics in the United States

Researchers studying other vector borne diseases have looked at landscape factors in endemic areas in an effort to learn what factors are associated with infection rate. Heartworm infections have been hypothesized to be more common in urban areas, although the actual reasons for this relationship are unstudied (Marks and Bloomfield 1998). Indeed, there is a lack of knowledge about the relationship between landscape types such as urban and rural and vector infection data in dog heartworm. Other epizootic pathogens have been studied in this context. For example, West Nile virus (WNV) is an invasive, zoonotic, epizootic pathogen in the United States. The abundance of the primary vectors of West Nile virus in the eastern United States, *Culex pipiens* and *Cx. restuans*, is positively correlated with human density, housing density, and urban land use, while negatively correlating with age of homes and amount of forested areas (Trawinski and Mackay 2010). In Georgia, songbirds collected on an urban-rural gradient revealed increased prevalence of antibodies to WNV in urban areas (Bradley et al. 2008). In Hawaii, an invasive species of mosquito, *Cx. quinquefasciatus,* transmits avian malaria to the resident birds (Reiter and LaPointe 2007). Researchers found that there was a higher prevalence of *Cx. quinquefasciatus* in mixed agricultural and residential areas and in areas with high levels of forest fragmentation, relative to birds that nested in intact forest (Reiter and La Pointe 2007). Birds that nested in national parks near these types of landscapes were at a high risk of infection due to the mobility of both the vector and the host.

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CHAPTER III

PREVALENCE OF DOG HEARTWORM, *DIROFILARIA IMMITIS*, IN COYOTES, *CANIS LATRANS*, IN OKLAHOMA AND NORTH TEXAS

Abstract

Dirofilaria immitis is a nematode parasite that causes a serious, fatal disease in domestic dogs and cats as well as wild canids, felids, and procyonids. The dog heartworm disease cycle includes *D. immitis,* the pathogen, mosquito vectors, and carnivorous mammals as definitive hosts of the disease. Although this disease can be prevented with the use of anthelminthic drugs, many dogs and cats remain at risk because they are not given adequate preventative medicine (Bowman 2009). In an effort to learn more about the sylvatic cycle of heartworm in Oklahoma, whole blood and serum samples were collected from 77 coyotes in rural areas in seven counties throughout the Oklahoma and Texas from January to March, 2010. Coyote carcasses were donated by the Oklahoma Department of Agriculture, Food, and Forestry USDA Wildlife Services and the Oklahoma Predator Hunters' Association. Of the 77 coyotes tested, 5 (6.5%) were positive for heartworm antigen. The distribution of infection showed a possible trend of higher infection in eastern than western areas, although the overall prevalence was low relative to studies from the eastern United States.

Introduction

Coyotes, *Canis latrans,* can be infected with *Dirofilaria immitis*, the causative agent of dog heartworm through the bite of an infected mosquito. Comparing studies of prevalence in coyotes suggests a strong influence of geography in determining prevalence, with studies in the eastern and midwestern United States demonstrating high prevalence. Of 24 coyotes collected along the Gulf Coast including Texas and Louisiana, 17, or 71 % of the coyotes were infected (Custer and Pence 1981). Forty-three percent of the coyotes collected in Florida were found to be heartworm positive (Foster et al. 2003). In rural Illinois, 16% of the coyotes tested were positive for heartworm infection (Nelson et al. 2003) (Table 3.1). On the other hand, studies from the West Coast of the United States have generally shown low rates of infection. In Washington state, researchers used cardiac dissection as a detection technique and found none of the 556 coyotes examined were infected (Foryet 2008). Sacks et al. collected coyotes at the California county level and found a wide range of prevalence rates, 0-25% (2004).

Although information from the east and west of the United States about wild canid infection rates is available there is no information about Oklahoma. There are data for domestic dogs, and Bowman et al. showed the prevalence of heartworm in domestic dogs that present at veterinarians in Oklahoma to be 2.1%, in the few counties with sufficient reporting (2009). The majority of the state did not have any data because of low reporting, making conclusions about local prevalence difficult. In addition, domestic dogs that show up at veterinarian offices likely represent a biased sample of the population of domestic dogs as a whole. One approach to gain a more complete understanding of heartworm infection in Oklahoma is to study the infection in *C. latrans* because coyote are untreated, likely have consistent opportunities for exposure, and distributed throughout this ecologically diverse state.

Oklahoma has eleven level three ecoregions spanning the state (Environmental Protection Agency 2000). These ecoregions include: the western high plains, southwestern tablelads, central great plains, flint hills, central Oklahoma/Texas plains, south central plains, Ouachita mountains, Arkansas valley, Boston mountains, Ozark mountains, and the central irregular plains. Due to the great variation in the landscape, Oklahoma is an excellent location to examine the relationship between local conditions and prevalence of heartworm in coyote.

The objective of this study was to determine the prevalence rate of heartworm infections in coyotes across the diverse landscapes of Oklahoma. This was accomplished through sampling coyotes killed on governmental and private hunts and assaying them for infection with dog heartworm. In addition to my focus on dog heartworm, I was also able to collect data from the coyotes on other common vector-borne infections.

Materials and Methods

Coyote Sampling

Coyote are killed in high numbers by three groups in Oklahoma: the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) wildlife service, the Oklahoma Predator Hunters' Association, and private wildlife control. The USDA and the Oklahoma Predator Hunters' Association donated harvested coyote carcasses for this research during the winter, January to March, of 2010. Additional coyote carcasses were collected opportunistically as road kill. All geographic data were collected using a global positioning system GPSmap76Cx, (Garmin company, Olathe, Kansas, USA).

The USDA-APHIS wildlife service conducts aerial hunts of cattle ranches during calving season (January to March) at the request of the ranchers because of a fear the coyotes will kill calves. Employees regularly collect over thirty coyotes at each hunt. After the coyotes are killed, the carcasses are collected for disposal. In 2010, I attended two USDA hunts: one on January 27 in

Craig County and one on March 17 on two separate cattle ranches in Okmulgee County. Once the carcasses were retrieved by USDA workers, I collected blood and tissue samples for *D. immitis* testing and examination for other parasites by collaborating researchers. Geographic data for all coyotes collected through this program, at each given hunt, is the approximate center of the ranch. I sampled 62 coyotes collected by USDA coyote hunts.

The Oklahoma Predator Hunters' Association (OPHA) has statewide and local hunts primarily in the winter. The association was founded to unite predator hunters from throughout Oklahoma to attend local hunts and hunting contests. The hunters usually keep only the skull or hide of the coyotes, so they were willing to donate the carcasses for research sampling. They attract the coyotes to their location using distress prey calls. In 2010, I attended two OPHA coyote hunts. Because the hunters were often unable to provide accurate kill location, the geographic data for these coyotes is the center of the county in which they were killed. I sampled 14 coyotes that were collected by private hunters through the OPHA.

Road kill opportunities provided additional coyote samples. Road kill coyotes were collected on a convenience and opportunity basis only. I sampled one road kill coyote in Payne County. From all three types of coyote collection opportunities, I was able to sample from five ecoregions including, the Arkansas valley, the central Great Plains, the central Oklahoma/Texas plains, the south central plains, and the central irregular plains.

Blood Collecting from Carcasses

Because the coyotes are dead and some may have been dead for several hours, blood samples were taken from the coyote hearts. I opened the ribcage and bisected the heart. Using a 3 ml syringe I extracted 3 ml of blood from each coyote and put it into a blood tube containing an anticoagulant, EDTA, and a blood tube without an anticoagulant. Additional muscle and skin samples were collected for collaborative research projects.

Blood testing

In the laboratory, the coagulated blood was centrifuged for ten minutes at 3500 rpm. The serum was removed using disposable pipets and put into microcentrifuge tubes. If antigen tests were not immediately available, the serum was frozen and recentrifuged before testing. Idexx Laboratories, Inc. (Westbrook, Maine) donated SNAP 4Dx tests which detect canine heartworm antigen, and *Anaplasma phagocytophilum*, *Borrelia burgdorferi,* and *Ehrlichia canis* antibody. Results of tests for all four diseases were recorded. Three drops of whole blood or serum was added to four drops of the provided conjugate. This solution was mixed by inversion and poured onto the test. When the liquid is absorbed across the test, the activator button must be fully compressed. The veterinarian must wait eight minutes for test results (IDEXX Laboratories, Inc.). The SNAP test has high sensitivity and specificity; for heartworm antigen, the test is 99.2% sensitive and 100% specific, for *A. phagocytophilum* the test is 99.1% sensitive and 100% specific, for *B. burgdorferi* the test is 98.8% sensitive and 100% specific, and for *E. canis* the test is 96.2% sensitive and 100% specific (IDEXX laboratories).

Results

Coyotes were collected from six Oklahoma counties and one Texas county, including 29 from Craig (36°45'N,95°08'W); six from Creek (35°50'N, 96°19'W); two from Logan (35°56'N, 97°31'W); 33 from Okmulgee (35°40'N, 95°58'W); two from Payne (36°08'N, 97°00'W); one from Roger Mills (35°37'N, 99°38'W); and four from Collingsworth (35°02'N, 100°20'W). Positive heartworm samples were obtained from coyotes collected in Creek, Okmulgee, Craig, and Collingsworth Counties (Figure 3.1). One female coyote of 29 samples from Craig County was infected, as were two males of six coyotes from Creek County, and one female of four from Collingsworth County (Table 3.2). There were no coyotes from the other counties positive for heartworm antigen. Therefore, the overall infection rate for heartworm was 6.49%.

In addition to testing for *D. immitis* antigen, the IDEXX snap tests also detects antibodies of *Anaplasma phagocytophilum, Borrelia burgdorferi,* and *Ehrlichia canis.* In the 77 Oklahoma and Texas coyotes, none were positive for *A. phagocytophilum* or *B. burgdorferi*. In Craig and Creek Counties, one male and one female were positive for *E. canis* (Figure 3.2). None of the other coyotes were infected with *E. canis.* Overall four of 77 coyotes were positive for *E. canis*, giving a statewide prevalence of 5.19% (Table 3.3). One female from Creek County was co-infected with both *D. immitis* and *E. canis.*

Discussion

Nationally, dog heartworm has been increasing its range in the last 30 years in domestic dogs, in spite of effective preventative treatment (Weinman and Garcia 1974, Pennington et al. 1970, Kocan 1976). Heartworm infections in domestic dogs in California were not diagnosed until the 1970s (Weinmann and Garcia 1974)and a recent national survey showed dog heartworm in domestic dogs throughout the continental United States (Bowman et al. 2009). At 3.9%, the southeastern states had the highest prevalence rate in the country while Oklahoma had a statewide prevalence of 2.1% (Bowman et al. 2009). Lincoln county in Oklahoma reported the highest infection rate , > 6.1%, in the state, however, many counties had fewer than ten test results, so information from those counties were not included in the analysis (Bowman et al. 2009). Based on the results of the 77 specimens I sampled, coyotes have a higher prevalence rate of heartworm than dogs tested at veterinary offices in Oklahoma (Bowman et al. 2009). This is intuitive because the dogs taken to the veterinarian are more likely to be on preventative than wild, unprotected coyotes.

The prevalence of *D. immitis* in coyotoes in rural areas of Oklahoma was low relative to data from eastern states, but similar to some studies in the western United States. Western states tend to have lower prevalence rates. Sacks et al. (2004) collected coyotes at the county level in

California and found a range of prevalence rates, 0-25%, very similar to what I found. Likewise, in Washington state, researchers used cardiac dissection as a detection technique and found none of the coyotes were infected (Foreyt 2008). On the other hand, eastern states have reported high levels of infection in coyotes. For example, of 24 coyotes collected along the Gulf Coast, 71 % were infected (Custer and Pence 1981). Forty-three percent of the coyotes collected in Florida were found to be heartworm positive (Foster et al. 2003). While every attempt was made to collect coyotes from as many ecoregions of the state as possible, only five were represented in this study. Additionally, low numbers of coyotes were collected from some of those ecoregions. Although it is impossible to make definitive conclusions about infection data with such low numbers, coyotes collected in eastern Oklahoma tended to have higher rates of infection than those collected in western Oklahoma, fitting the general pattern found comparing previous, geographically diverse studies.

Infection rates can be dynamic, contributing to variation in observations. For example, age structure of the coyotes may contribute to variation in infection rates. In Illinois, coyotes less than six months old had an 8.7 % infection rate while those over 3.5 years had 40.4 % infection rate (Nelson et al. 2003). I only examined adult coyotes (older than one year), and did not age coyotes, so it is possible our sample was not representative of all coyotes, and may contribute to the low prevalence rate. In addition, *D.immitis* may be expanding its range in wild canids. For example, in the early 1990s, heartworm infections in wild canids in California were low, but increased to 91% by 1996 in a small sample of 23 coyotes collected in California (Sacks 1998), suggesting an invasion of California. If this invasive process is ongoing, the prevalence of dog heartworm in Oklahoma coyotes may increase in the future.

Another source of variation may be due to the different methods used by researchers to detect *D. immitis*. Cardiac dissection is used to visually assess whether or not there is a heartworm infection. There is some concern that using only cardiac dissection might yield many false negatives. If there are few heartworms, or the worms are small, it may be difficult to accurately assess using cardiac dissection (Foreyt 2008). SNAP antigen tests are the standard test used by veterinarians for testing patients for heartworm. SNAP tests only work if there are reproductively mature female worms (Bowman and Atkins 2009). My coyotes were all adults and were collected in the winter, so if they were yearlings, and infected the previous spring or summer, the infection should have been patent at time of harvest.

The relatively low prevalence rate in coyotes collected in rural Oklahoma compared to the Gulf Coast states and Midwest could be caused by differences in the vector or definitive host natural history and biology. The coyotes were collected primarily from rural areas where standing water is limited to stock tanks and ponds. Many mosquitoes rely on simple aquatic environments for breeding , so an environment without diverse aquatic habiats would limit the diversity and abundance of vectors (Laird 1998). Furthermore, the vector species composition is different from one part of the country or one landscape type to another. For example, one species of mosquito, *Ades. Albopictus* Skuse, that has been implicated as being an important vector of heartworm in other parts of the world is not successful in rural Oklahoma (Cancrini et al .2003, Lee et al. 2007, Licitra et al. 2010, Paras 2011 this work). Indeed, work on mosquito vectors of *D. immitis* in Payne County, OK suggests that urban areas have much higher rate of active transmission than rural areas, based upon vector infection rates (Paras, 2011). I did not sample the mosquitoes at each site I collected coyotes, so the effects of variation in vector assemblage can only be speculated upon. A further study in which vectors are assessed at each collection site would help clarify if the variation in heartworm in Oklahoma can be attributed the vectors.

Host natural history may also contribute to the pattern. Coyotes in rural environments had smaller home ranges, of 4.3 to 4.7 sq km, than coyotes in urban locations 7.3 sq km (Andelt 1985, Gehrt 2007). The larger home range of urban coyotes provides more opportunities for them to come into contact with other definitive hosts, such as domestic dogs and infected mosquito vectors. The rural coyotes that I sampled may not have had as much interaction or overlap of home range as urban coyotes would. Small home ranges in rural environments may also limit exposure to domestic dogs, as domestic dog density is also lower in rural than urban areas (Paras, 2011, this work). This could account for the lower infection rate found in the Oklahoma rural coyotes, 6.5%, compared to coyotes living in Chicago, IL, 41% (Gehrt unpublished data, personal communication).

Due to the low prevalence rate of heartworm infections in rural Oklahoma coyotes and the suggestion from the literature and my vector study that urban areas may have a greater risk of heartworm transmission, future studies should compare the prevalence of heartworm in coyotes living in Tulsa or Oklahoma City to the coyotes I sampled. Based on studies conducted in Illinois, coyotes from rural areas of the state had a 16% prevalence rate, while those in Chicago had a 41% prevalence rate (Nelson et al. 2003, Gehrt unpublished data). Researchers in Oklahoma could also do a concentrated study about the infection rates found in domestic dogs and cats brought to animal shelters, which, like coyotes, may be less likely to be protected with prophylactic anthelminthics.

Table 3.1 Review of infection rates of coyotes with *D. immitis* in published studies

Location	Percent infected	Number infected	Number Collected	Citation
Missouri	40.4%	119	293	Wixsom et al. 1991
Gulf Coast	71%	17	24	Custer and Pence 1981
Washington	0%	0	556	Foreyt 2008
California	0-25%	N/A	1703	Sacks et al. 2004
Illinois	16%	147	920	Nelson et al. 2003
Florida	43%	11	26	Foster et al. 2003

Table 3.2 *D. immitis* prevalence rate in coyotes by Oklahoma and Texas county

County	Percent infected	Proportion infected	Sex of coyotes
Craig	3.4 %	1/29	Female
Payne	0 %	0/2	N/A
Roger Mills	0 %	0/1	N/A
Logan	0 %	0/2	N/A
Creek	33 %	2/6	Male, Male
Collingsworth	25 %	1/4	Female
Okmulgee	3.03 %	1/33	Female
Total	6.49 %	5/77	3 Female, 2 Male

Table 3.3. *E. canis* prevalence rate in coyotes by Oklahoma and Texas county

County	Percent infected	Proportion infected	Sex of coyotes
Craig	6.9%	2/29	Male, Female
Payne	0%	0/2	N/A
Roger Mills	0%	0/1	N/A
Logan	0%	0/2	N/A
Creek	33%	2/6	Male, Female
Collingsworth	0%	0/4	N/A
Okmulgee	0%	0/33	N/A
Total	5.19%	4/77	2 Male, 2 Female

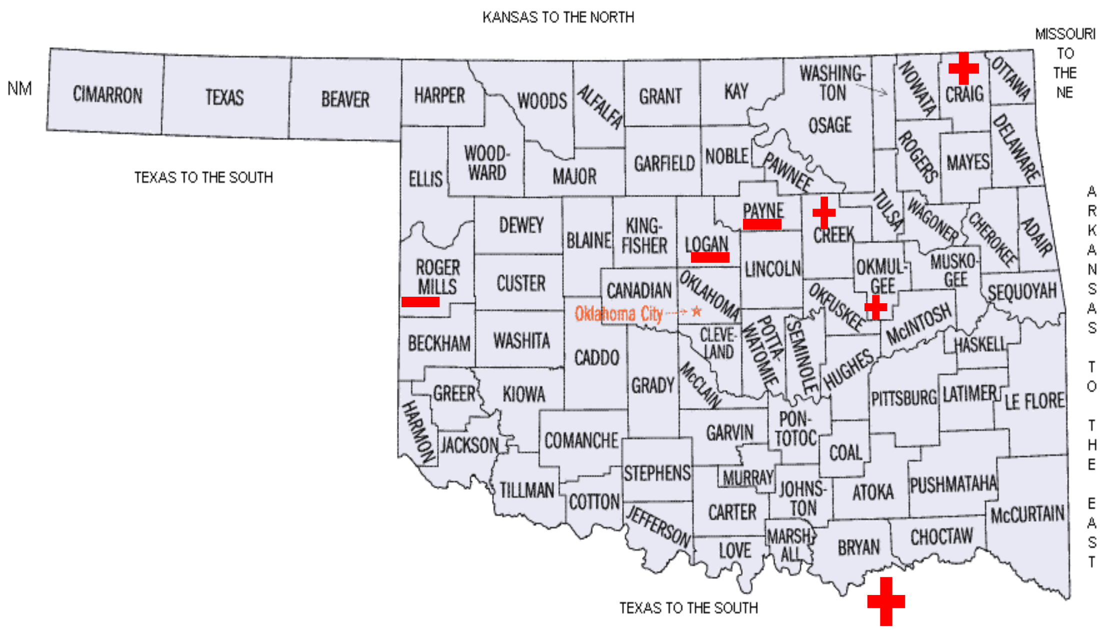

Figure 3.1 Map of Oklahoma counties indicating where coyotes were collected and which counties had heartworm positive samples (http://sdwis.deq.state.ok.us/DWW/Maps/Map_Template.jsp)

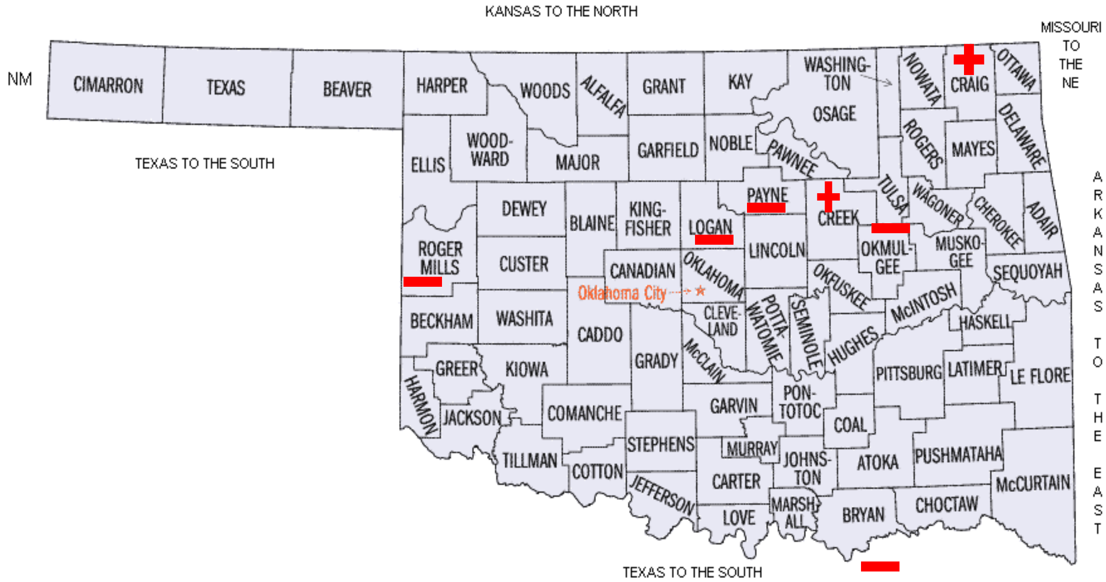

Figure 3.2. Map of Oklahoma counties indicating where coyotes were collected and which counties had *Ehrlichia canis* positive samples (http://sdwis.deq.state.ok.us/DWW/Maps/Map_Template.jsp).

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CHAPTER IV

INCRIMINATION OF MOSQUITO VECTORS OF DOG HEARTWORM IN RURAL AND URBAN SITES IN PAYNE COUNTY, OKLAHOMA

Abstract

Dirofilaria immitis Leidy is a mosquito-borne nematode that causes a serious, fatal disease in dogs and cats. Although this disease can be prevented with the use of anthelminthic drugs, many dogs and cats remain at risk because they are not given adequate preventative medicine. Another approach to prevention is control of important vector species, requiring local vector incrimination studies.

There were two main objectives of this study. The first was to evaluate the relationship between landscape and social factors and the number and species of heartworm positive mosquitoes. The second was to determine which species of mosquitoes trapped are infected with *D. immitis* in Payne County, with a specific focus on the importance of the invasive Asian tiger mosquito, *Aedes albopictus* (Skuse).

To achieve these objectives, mosquitoes were collected from May to November, 2010, from 16 rural and 16 urban locations in Payne County using three trapping methods: resting boxes, carbon dioxide traps, and BG sentinel traps. Urban collected mosquitoes had significantly higher maximum likelihood of infection, 2.59%, than rural collected

mosquitoes, 0.97% (P<0.05). Two species, *Aedes albopictus* and *Psorophora columbiae*, were incriminated as important vectors if heartworm in Payne County. Considering the higher infection rate and the importance of *A. albopictus* in urban areas, control through elimination of container breeding habitats in the peridomestic environment may be a good approach to limiting transmission of *D. immitis* in urban areas in the southern Midwest.

Introduction

The prevalence of vector borne diseases in the vector or host can be associated with landscape factors in endemic areas. Urbanization and habitat fragmentation have been shown to be important landscape factors for predicting the spread epizootic, mosquito-borne disease (Trawinski and Mackay 2010, Reiter and LaPointe 2007, Siers et al. 2010, Bradley et al. 2008). Dog heartworm infection with *Dirofilaria immitis* Leidy, is a common vector-borne disease throughout the United States that provides an opportunity to examine the effects of landscape factors on transmission of a mosquito-borne pathogen.

Dirofilaria immitis causes a serious, fatal disease in dogs and cats. Although this disease can be prevented with the use of anthelminthic drugs, many dogs and cats remain at risk because they are not given adequate preventative medicine (Bowman 2009). This disease is important to pet owners due to the devastating effects on untreated animals, and much of the research conducted has looked at infected definitive hosts only. The traditional approach to disease control has been to protect animals with a prophylactic ivermectin, milbemycin oxime, selamectin, or moxidectin regime. Prevention is critical, because once an infection occurs treatment is much more difficult and expensive (Bowman 2009). However, the emergence of resistance to ivermectin in other filarids raises the possibility of resistance in *D. immitis*, which would make current control strategies untenable (Prichard 2005)..

Another approach to prevention is control of the mosquito vectors. Dog heartworm is spread by the bite of over 60 species of mosquitoes worldwide (Ludlam et al 1970). This broad vector range makes vector incrimination important in any given location, and may allow general conclusions about the interaction of vectors with landscape or socioeconomic factors. Vector incrimination is critical to pest control strategies because targeting specific vectors can be an effective and economical approach to disease prevention.

There have been numerous studies in the United States over the last forty years that have demonstrated a variety of other potential vectors, including mosquitoes in the genera *Anopheles*, *Psorophora*, *Culex* and *Aedes* (Eldridge and Edman 2000). Local vector communities vary in which species are likely to be important vectors. For example, researchers conducted a study of the presence of L3 larvae in mosquitoes collected from Gainesville, Florida, Bartow, Florida, and Baton Rouge, Louisiana (Watts et al. 2001). In Gainesville, Florida *Aedes canadensis, Ae. vexans, Anopheles crucians, Ae. infirmatus*, *Culex nigripalpus*, and *Psorophora ferox* were all found to be positive for *D. immitis. Anopheles crucians* had the highest rates of infection with 0.2% (Watts et al. 2001). In Bartow, Florida only *Ae. vexans* were positive with heartworm at a rate of 2% (Watts et al. 2001). The researchers found positive pools of *Ae. vexans*, *An. quadrimaculatus,* and *Ps. columbiae* in Baton Rouge, Louisiana (Watts et al. 2001). More recent studies in Georgia, researchers found three species of mosquitoes to be heartworm positive including, *Ae. albopictus, An. punctipennis*, and *An. crucians* (Licitra et al. 2010).

The only work on vector incrimination for dog heartworm in Oklahoma was conducted nearly thirty years ago. This vector study work incriminated *Aedes trivatattus* (Coquillet) and *Culex erraticus* (Dyar and Knab) as the important *D. immitis* vectors in Oklahoma based on mosquito feeding habits, numbers of mosquitoes, and ability to transmit heartworm (Afolabi et al. 1988, 1989). However, this study was conducted at a single site in Payne County, using a dog infected with *D. immitis* as bait, preventing any examination of underlying landscape or socioeconomic factors that may contribute to dog heartworm transmission. In addition, the vector community in Oklahoma has changed since this study was in the late 1980s. Therefore, the most important and influential vectors may have shifted since Afolabi et al's (1989) study.

A dramatic change in the vector community of Oklahoma has been the invasion by the Asian tiger mosquito, *Ae.s albopictus* (Hawley 1988). This mosquito is a container-breeding, synanthropic mosquito originally found in East Asia (Hawley, 1988). It is an important vector of numerous human pathogens, including dengue virus, chikungunya virus, and the filarid nematode that causes human filariasis, *Burgia malayi* (Gratz, 2004). Throughout its native home range, *Ae. albopictus* has been shown to be a vector of heartworm. Although found to have lower infection rates than *Cx. quinquefasciatus* in Taiwan, *Ae. albopictus* is an important vector on this island nation (Lai et al. 2001). *Aedes. albopictus* is known to be an important vector of *D. immitis* larvae in Singapore, Japan, China and Korea (Chellappah and Chellappah 1968, Konishi 1989, Lai et al. 2000, Lee et al. 2007). In laboratory experiments, *Ae. albopictus* maintained an average of 20.7 *D. immitis* larvae and up to 51 larvae for up to twelve days post-infection, and 99.4% contained at least one maturing larvae, indicating that *Ae. albopictus* is a competent vector of dog heartworm (Kartman 1953). The potential for the global invasion of this mosquito to contribute to the spread of disease has been suggested (Juliano and Lounibos, 2005), although empirical evidence has been limited. In Italy, which *Ae. albopictus* invaded in the 1990s, this species has been shown to be an effective and important vector of heartworm (Cancrini et al 2003).Furthermore, the spread of *D. immitis* from northern into southern areas of Italy has implicated *Ae. albopictus* as the driving force in this change in dog heartworm distribution (Otranto et al. 2009). However, the importance of *Ae. albopictus* in dog heartworm epidemiology in North America is speculated, but not well documented (Apperson et al. 1989, Watts et al. 2001). More recently, in a Georgia study, *Ae. albopictus* was found to have the highest infection rate, 2.3%, of the species collected (Licitra et al. 2010). Consequently, *Aedes albopictus* may contribute to the pattern of disease at a local level.

Payne County was selected for the study area due to its location. The city of Stillwater, the major urban area within Payne County, was used for urban trapping and agricultural land outside of the city limits was used for rural trapping. Stillwater is an ideal urban location because it is located approximately 100 km from the nearest large urban center, and thus represents an urban patch in a rural matrix. This reduces the chance that other urban areas outside of the study area might affect the results of the study.

There are two objectives of this study. The first is to evaluate the relationship between landscape types, specific landscape characteristics and social factors and the number and species of heartworm positive mosquitoes. Landscape and social factors include urban versus rural environment, housing density, tree density, age of neighborhood, socioeconomic status, available larval habitat, and host density. The second objective is to determine which species of mosquitoes are infected with *D. immitis* in Payne County and therefore likely to be important vectors, with a specific focus on the importance of the invasive *Ae. albopictus* as a vector of *D. immitis* in Payne County. The hypothesis for the first objective is that landscape and social factors affect mosquito infection rates of *D. immitis.* I predicted that landscape factors will correlate with the infection rate in mosquitoes and there will be differences in the two landscape types, urban and rural. The hypothesis for the second objective is that different mosquito species are important now compared to 30 years ago (Afolabi et al. 1989). I predicted that since the species composition may have changed alternate species will be more important vectors. Specifically, I predicted that *Ae. ablopictus* will be one of the most important vectors of heartworm in Payne County.

Materials and Methods

Mosquito Sampling

I collected mosquitoes between May, 2010 and November, 2010 in Payne County. Sixteen urban locations, eight cedar rural, and eight hardwood forest rural sites within Payne County were selected to trap mosquitoes (Figure 4.1 and 4.2). Selection of a trapping site was based on: the ability of researchers to reach the site; seclusion, so that the traps will not be stolen; and presence of adequate shade so the temperature is cool enough for mosquitoes to utilize the resting box traps. I was not able to randomly select sampling sites due to the constraints of mosquito biology. However, the large number of sites in each landscape type (urban and rural) likely captured the variation in the landscape. Sites were located at least 300 m apart, which reduces that chance that mosquitoes will fly between sites, being farther than the mean dispersal distance for a number of mosquito species (Reisen et al. 1991, Marcel De Freitas et al. 2007) Therefore, I considered each site as independent of one another. At each site, three resting boxes were placed within 10 m of one another. Resting traps consist of a dark-colored 30 gallon plastic box placed upside down with gaps cut in the bottom of the box. The gaps allow mosquitoes to enter the boxes as a refuge during the day. I visited each site weekly with a backpack aspirator and collected from each resting trap from May to November, 2010. In addition, natural refugia around each site were aspirated for 10 minutes after each resting trap had been collected. I used resting traps and area aspiration because they are a less biased trapping method than host seeking or gravid traps, and capture a greater diversity of mosquito species (Service, 1993) and most of the mosquitoes that land in resting traps have taken a blood meal (Kweka and Mahande 2009). Trapping blood-fed mosquitoes is advantageous because the microfilariae are ingested when the vector feeds on an infected host. Due to low numbers of mosquitoes collected via the resting box trapping method, carbon dioxide traps and BG Sentinel traps were set at the same locations to add volume to the mosquito samples. At the urban sites, carbon dioxide traps were run monthly and BG sentinel traps were set twice during the trial period. Carbon dioxide and BG sentinel traps were chosen because they attract mosquitoes which allowed me to supplement low numbers collected through the resting box method.

Collected mosquitoes were brought back to the laboratory, where they were identified using Darsie and Ward (2005). Mosquitoes were placed by species in individual microcentrifuge tubes. The mosquitoes were speciated and separated into pools of no more than 20 based on trapping location. All trapping methods, resting box, carbon dioxide, and BG sentinel traps were combined to create pools. Due to low numbers of some species of mosquitoes it was impossible to maintain temporal data for all pools. *Culex restuans* and *Cx quinquefasciatus,* and *Ps. ferox, Ps. longipalpus,* and *Ps. horrida* were pooled together due to the level of difficulty of differentiating among these species.

Detection of* <u>**Dirofilaria immitis**</u> *in Mosquitoes

The mosquitoes were tested for the presence of *D. immits* with real-time polymerase chain reaction (rt-PCR). The heads of the mosquitoes were tested because the larvae must be found in the head to be certain of the mosquito's vector competence. Pools of no more than 20 mosquitoes of the same species, from the same location were tested in one reaction. DNA was extracted using Qiagen QiAMP mini kits as per the manufacturer instructions (Qiagen Hilden, Germany). Blood from a *Dirofilaria immitis* infected dog was used as a positive control for unknown samples.

Primers were developed using genetic sequences provided by Genbank. Gene regions selected included cytochrome B, CN-49 16S, NADH dehydrogenase subunit 1, cytochrome C oxidase subunit, and *Culex pipiens quinquefasciatus* actin as a control. The primers were tested using Primer 3 (v 0.4.0) technology for accurate melting temperature 55-65° C and GC content 40-60%. Using IDT integrated DNA oligoanalyze 3.1, the primers were analyzed for self-dimers, hairpins, and hetero-dimers. To determine the best primer set, two cytochrome B primer sets, two NADH dehydrogenase subunit 1 primer sets, and one actin primer set were individually added to mosquito DNA combined with known heartworm positive canine blood DNA as well as uninfected mosquito DNA. The polymerase chain reaction contained dH2O 17.0 µl, 10x Taq buffer 2.5 µl, MgCl2 2.5 µl, 10 mM dNTP 0.5 µl, 10 mM forward primer 0.5 µl, 10mM reverse primer 0.5 µl, Taq polymerase 0.5 µl, and template DNA 1.0 µl. The PCR protocol was 95° C ten minutes, repeating 30 cycles of 94° C one minute, 50° C one minute, 72° C for one minute, and 72° C for ten minutes at the end. The gel electrophoresis protocol was 10 µl DNA and 2 µl loading dye at 50 V for approximately 40 minutes. Based on the results of this preliminary work, the selected primers for detection included a cytochrome B sequence (forward GGCTATTGGTTGAAGGATGG, reverse TGTCAGGAACAGAACGCAAA). Mosquito actin primers were also developed to be used as controls in the reactions (forward CAAGATTCAGCTGCCGTACA, reverse CAAACTCGCCAACATCTCCT).

The mosquito DNA was extracted using Qiagen minicamp DNA kits. The samples were prepared using the tissue protocols according to the manufacturer's instructions. Following extraction, the DNA was prepared for RT-PCR using 12.5 µl SYBR green, MgCl2 2.5 µl, 10 mM DNTP, 0.5 µl 10 mM forward primer, 0.5 µl 10 mM reverse primer, 0.5 µl, template DNA 1.0 µl, and dH2O 7.5 µl. The program for DNA amplification was 50° C two minutes, 95° C 15 minutes, and 40 cycles of 95° C for 15 seconds and 50° C for one minute.

I did not attempt to quantify the number of L3 larvae in each pool. I tested only for presence/absence of the pathogen. Each plate had positive controls of DNA from canine blood infected with heartworm combined with mosquito DNA. For my negative controls, I used uninfected canine blood DNA, known uninfected mosquito DNA, and water. Samples were determined to be positive if the critical threshold value was reached before 35 cycles.

Quantification of Landscape and Social Factors

To understand, quantify, and qualify variation between sites, a number of landscape and social factors were identified as potential influences on the number of mosquitoes collected in an area and the likelihood of finding dog heartworm positive mosquitoes. Surrounding each mosquito trapping site in Payne County, a nine block or equivalent area, (~360 m^2) was walked and each of the landscape factors were counted and assessed

I collected data on the number of residential buildings, number of trees greater than 3 m tall, and junk. Each home, other buildings, and trees were counted. The junk index rating was created on a scale from 0-5, with 0 being no trash and 5 being total contamination of yard (Table 4.1). The junk items did not have to be trash, but any potential item holding stagnant or dirty water that might serve as a mosquito larval habitat, or provide refuge for adult mosquitoes (e.g., water bowls, swimming pools, tires).

While walking around the neighborhood, the number of outdoor dogs and indoor dogs were counted separately. Additionally, homes that had evidence of dogs such as water bowls, dog houses, etc., were also recorded. The breed of dogs observed was recorded if possible. If it was impossible to determine from observation, an estimated size and mixed breed was recorded. Assessment of number of wild canids was not possible.

Socioeconomic variables were determined using public records. The property value (used as a proxy for socioeconomic status)and age of the neighborhood were found by looking at the average property value and age of the ten closest homes to the trap location in the neighborhood available on www.zillow.com (January 4, 2011).

Landscape factors were assessed once per summer between 6:00 and 8:00 PM on week-nights. This time and day range was selected because it coincides with when people were typically at home and had their dogs outside. This allowed for the most accurate count of dogs in a neighborhood. Also, many mosquito species feed in a crepuscular time frame, so this time also accurately predicted the number of dogs that are likely to be bitten. Temperature and humidity were assessed using iButtons© (Maxim Corporation, Houston, TX) twice during the summer. For each measurement date, the iButtons© were left at the site for one week and average temperature and humidity during that time was recorded.

Statistical analysis

To evaluate differences between urban and rural settings, I used t-tests and Mann Whitney non-parametric rank tests. Spearman correlations were used to examine correlations between infection rate by site and each landscape and social factor. The Center for Disease Control developed a program to determine the maximum likelihood of infection estimates of infection rates for disease detection in pools of mosquitoes. I pooled mosquitoes based on species and site. Pools were made up of at least one and no more than 20 mosquitoes. This test generates a maximum likelihood of infection based on pool size and number of pools tested and provides 95% confidence intervals. When these intervals do not overlap then we can be confident (P<0.05) that the infection rates are significantly different (Biggerstaff 2009).

Results

Mosquito trapping

Mosquitoes were collected in all trapping sites throughout the summer. Significantly more mosquitoes were collected in urban resting boxes (0.651 ± 0.342) than rural resting boxes (0.006 ± 0.006; Mann Whitney test, $U_{(16,16)}$ = 221.5, p = 0.286)) throughout the entire trapping season, May-November, 2010 (Figure 4.3). Three carbon dioxide trapping dates the weeks of July 13, August 17, and August 24, 2010, coincided between urban and rural sites. There were no differences in number of mosquitoes captured by CO_2 traps between the two landscape types on any of the dates (rural 18.43 ± 2.67, urban 50.81 ± 24.33Mann Whitney test $U_{(16,16)}$ = 249, p = 0.5842; rural 5.69 ± 1.56, urban 14.69 ± 4.38 Mann Whitney test $U_{(16,16)}$ = 218.5, p=0.0887; and rural 15.57 ± 4.18, urban 7.77 ± 1.94 Mann Whitney $U_{(16,16)} = 202$, $p = 0.5079$)(Figure 4.4). There was tremendous variation between replicates within urban and rural landscapes which accounts for the lack of difference between the two landscape types. *Aedes albopictus* were collected in high numbers, 819 individuals, from all trapping methods in urban locations.

Infection with **<u>D. immitis</u>**

Heartworm positive mosquitoes were collected in nine of the sixteen rural locations and six of the sixteen urban locations. The maximum likelihood of infection for all species in urban sites was 2.59% (95% CI 1.9-3.44%) (Table 4.2) (Figure 4.5). *Aedes albopictus* in urban locations had a maximum likelihood of infection of 1.69% (95% CI 0.95-2.81) (Figure 4.5). In rural locations, the maximum likelihood of infection for all species was 0.97% (95% CI 0.64-1.4%). Although collected in lower numbers, 191 individuals, in rural locations, *Ae. albopictus* did have a maximum likelihood of infection of 1.56%, indicating that they are transmitting the disease in both landscape types (Table 4.3). *Psorophora columbiae* were collected equally in rural, 208 individuals, and urban, 206 individuals locations. In the rural landscape, the maximum likelihood of infection for *Ps. columbiae* was 1.48% (95% CI 0.41-3.97%) and in urban locations 1.88% (95% CI 0.67-4.32%) (Table 4.4). While some other species of mosquitoes have high maximum likelihood of infection rates, the number of individuals collected and the number of pools tested are too low to make any valid conclusions about their importance in the heartworm transmission cycle in Payne County. There were no significant correlations between prevalence of heartworm in the mosquitoes and any of the landscape factors collected. Although there were no significant differences by site, there were differences in landscape and social factors by the landscape types urban and rural. Significantly more domestic dogs and houses were observed in urbanrural dogs than rural sites (rural 0.4375 dogs ± 0.1819, urban 7.4375 dogs ± 1.144, $p<0.0001$, t-test t= -6.04) (rural 1.3125 houses ± 0.723, urban 46.25 houses ± 0.0514, $p<0.0001$, t-test t= -7.52) (Figure 4.6 and 4.7). The urban landscape had a trend towards a higher junk index than the rural landscape (rural 0.0807 junk index rating ± 0.063, urban 0.223 junk index rating ± 0.0514 p=0.0953t-test t= -1.72) (Figure 4.8). The houses in the urban landscape were significantly older than those in the rural sites (rural 32.4 years old ± 4.957, urban 50.1 years old ± 4.886 p=0.0486, t-test t= -2.11) (Figure 4.9). The temperature in July was significantly warmer in urban locations, but significantly cooler in September (rural 25.1° C ± 0.123, urban 25.7° C ± .159 p=0.0073t-test t= -2.90) (rural 25.3° C ± 0.124, urban 24.5° C ± 0.186 p=.0005t-test t= 3.92) (Figure 4.10 and 4.11). The humidity in urban locations in September was significantly higher than the rural sites (rural 80.8 %RH ± 0.675, urban 89.9 %RH ± 1.134 p<.0001t-test -6.93) (Figure 4.12). There were no significant differences in the average number of trees, average cost of homes, or the humidity in July, 2010.

Discussion

In this study the objectives included determining which mosquito vectors were the most important in transmission of *D. immitis*, with specific reference to *Ae. albopictus*, and what landscape factors were important in the presence or absence of heartworm infected mosquitoes. I found several species were likely important vectors of dog heartworm in Oklahoma, and that the invasive *Ae. albopictus* was both abundant and a likely vector in urban areas. Although landscape factors did not correlate with infected mosquitoes at the level of each site, there were marked differences in the measured factors and infected mosquitoes between urban and rural sites.

Throughout my study, I collected seven genera and 25 species of mosquitoes. Of these 25 species, 15 were found to be infected with *D. immitis*. *Aedes albopictus* and *Ps. columbiae* appear to be the most important vectors of heartworm based upon their abundance and likelihood of being infected in urban and rural locations, respectively. Other species of mosquitoes had high maximum likelihood of infection rates, but the number of individuals collected and the number of pools tested were too low to make valid conclusions about their importance in the heartworm transmission cycle in Payne County.

The fact that *Ae. albopictus* is an important vector of heartworm disease in urban areas fits with studies throughout the native and invasive range of the species. Throughout its native home range, including Singapore, Japan, China, and South Korea, *Ae. albopictus* is known to be an important vector of *D. immitis* larvae (Chellappah and Chellappah 1968, Konishi 1989, Lai et al. 2000, Lee et al. 2007). Although found to have lower infection rates than *Cx. quinquefasciatus* in Taiwan, *Ae. albopictus* is an important vector on this island nation (Lai et al. 2001).

In its introduced range *Ae. albopictus* also appears to be an important vector. The spread of *D. immitis* in Italy from northern regions into southern areas has been linked to the invasion of *Ae albopictus* (Otranto et al. 2009). Although not directly linked to the spread of dog heartworm, *Ae. albopictus* in the United States has been suggested to be a vector (Apperson etal. in 1989, Nayar et al. in 1999). More current research in Georgia, USA, showed that, *Ae. albopictus* was found to have the highest infection rate, 2.3%, of the species collected throughout the study area (Licitra et al. 2010).

While important vectors were incriminated in my study, there were no correlations between any of the landscape or social factors that I collected and infection rate by site. However, there were differences between urban and rural landscapes. Urban sites had significantly higher rates of infection, number of dogs and houses, average temperature in July and average relative humidity in September. There were more mosquitoes collected in urban resting boxes and a trend towards higher average junk index rating in urban locations. The rural sites had significantly higher average temperature in September.

I observed little difference in overall mosquito abundance in urban versus rural areas, although there were differences in species composition. As previously mentioned, *Ae. albopictus* was much more abundant in urban areas, where it was an important vector of *D. immitis*. *Aedes albopictus* is often found in artificial containers in the peridomestic environment (Hawley, 1988), and urban areas did have a marginally higher junk index, and, perhaps more importantly, many more houses.

Because the temperatures were higher in each landscape type depending upon time of year, it is difficult to make a conclusion about the importance of temperature on infection. The higher humidity in the urban areas could be a result of the high temperatures and dry conditions in late summer and result from people watering their gardens and lawns. Over-watering and creating pools of water in the grass creates an ideal breeding site for *Ps. columbiae* which was the other species found to be an important vector of heartworm in both urban and rural landscapes. Watering may also fill container habitats favored by *Ae. albopictus.*

Significant differences were seen between the infection rate in urban and rural landscapes. Mosquitoes in urban locations were more likely to be infected with heartworm than those in rural locations. This could be due a variety of differences between urban and rural areas, including the number of available hosts, abundance of vectors, availability of larval habitat for vectors, and other biotic or abiotic conditions. While no attempt was made to quantify the number of alternate hosts in either landscape type, there were significantly more domestic dogs observed in urban than rural sites. This high number of potential definitive hosts may contribute to the higher prevalence of infected mosquitoes. This is intuitive because as the number of hosts increases, the opportunity for disease infection increases. The increase in disease in urban areas is not unique to heartworm. Researchers studying other mosquito-borne diseases found that *Cx. quinquefasciatus* in mixed agricultural and residential areas and in areas with high levels of forest fragmentation had higher rates of avian malaria (Reiter and La Pointe 2007). Birds that nested in national parks near these types of landscapes were at a high risk of infection due to the mobility of both the vector and the host. Researchers in Georgia, USA found a correlation between cases of West

Nile virus and the urban rural gradient. As the area become more urbanized, the birds were more likely to be infected (Bradley et al. 2008)

Because *Ae. albopictus* is an important vector of heartworm in urban areas of Oklahoma, control of this vector may be important in reducing transmission to definitive hosts. *Aedes albopictus* are container breeding mosquitoes, so reducing the number of potential larval habitat areas reduces the ability of the species to oviposit and develop near dogs. Any trash articles that are present in a yard should be disposed of properly. Old tires, if not disposed, should be kept under cover. Bird baths, kiddie pools, and other desirable lawn items should be kept clean. The water should be changed on a regular basis to prevent mosquito larvae from developing. By reducing the containers that are important larval habitat for *Ae. albopictus*, the population may be reduced and potentially less heartworm will be transmitted by this species (Richards et al. 2008). Community-based efforts have been effective at controlling *Aedes aegypti* in dengue, a pathogen-vector system with a similar ecology to dog heartworm, in endemic areas (Erlanger, et al. 2008).

Although there was a relationship between landscape type and infection, I did not find any significant correlations between individual sites infection rates and any of the landscape and social factors collected. Future work should be conducted to look at the same factors on a different spatial scale. Perhaps I looked at too large of any area around the trapping site and that infected mosquitoes are not influenced by factors at that large of a scale.

Additionally, it is possible that some of the information collected might be incomplete because it was observational. Although attempts were made to provide accurate counts, definitive host counts may have been inaccurate because some owners keep their dogs inside. Future researchers could survey residents in the trapping area to determine detailed information including number of dogs, breed of dogs, whether or not they are given preventative treatment for heartworm, and how long and during what hours dogs are kept outside. This information could provide researchers with more knowledge about the study area than observation alone.

There were problems associated with the study that may have influenced differences between landscape types. Significantly more mosquitoes were collected from urban resting boxes than rural resting boxes. Because resting boxes are ideal for collecting blood fed mosquitoes, there may have been a bias in collecting more urban blood fed mosquitoes than rural blood fed mosquitoes (Kweka and Mahande 2009). In the future, researchers should consider trapping more frequently with carbon dioxide traps to increase the number of individuals tested.

The mosquito information is important on a local level because no research has been done on heartworm vectors for thirty years in Oklahoma. During that time, the species composition has changed (Moore et al. 1999) and *Ae. albopictus* have become important vectors. This study was important on a larger scale because the invasion of *Ae. albopictus* is not unique to Oklahoma. The species has spread throughout the Southern United States, South America, Europe, and parts of Africa. The study is also relevant to other parts of the world because we found a relationship between urban areas and higher infection rates in mosquitoes. Other studies have seen an increased prevalence of heartworm in urban versus rural wild canids, but no vector studies had previously been conducted to corroborate these data (Marks and Bloomfield 1998, Gehrt 2007).

Table 4.1. Junk index rating determination- junk index rating used to rank sites by the amount of anthropogenic created oviposition sites.

Index rating	Description
0	No visible trash in yard or outside home
1	Less than three pieces of potential larval habitat
2	Between four to seven pieces of potential larval habitat
3	Between eight to twelve pieces of potential larval habitat
4	Between thirteen to seventeen pieces of potential larval habitat
5	Over eighteen pieces of potential larval habitat

Table 4.2. The number of individuals, pools, positive pools, and infection rate with *D. immitis* for mosquitoes collected in urban and rural sites

Species	# indi rural	# pools rural	# pos pools rural	Lower limit	Upper limit	MIR	# indi urban	# pools urban	# pos pools urban	Lower limit	Upper limit	MIR
Ae al	191	27	3	0.42	4.11	1.56	819	90	13	0.95	2.81	1.69
Ae ep	5	5	0	0	0	0	0	0	0	0	0	0
Ae tr	26	8	1	0.23	14.78	3.31	13	8	2	3.03	38.09	14.44
Ae ve	572	37	3	0.04	0.39	0.15	30	10	1	0.2	15.34	3.32
An pu	11	10	1	0.53	36.34	9.09	0	0	0	0	0	0
An qu	28	13	1	0.21	15.64	3.47	14	9	2	2.86	44.51	15.71
Cq pe	28	5	0	0	0	0	3	3	0	0	0	0
Cs in	35	13	0	0	0	0	0	0	0	0	0	0
Cx co	0	0	0	0	0	0	4	3	0	0	0	0
Cx er	657	39	2	0.06	1.01	0.31	75	13	2	0.08	6.03	1.28
Cx pe	7	3	0	0	0	0	0	0	0	0	0	0
Cx qu/re	132	20	2	0.28	4.67	1.47	46	12	2	0.87	12.2	4.07
Cx sa	8	2	0	0	0	0	15	6	1	0.4	26.32	6.32
Cx ta	507	31	2	0.07	1.34	0.41	1	1	0	0	0	0
Oc at	5	2	1	1.42	82.05	22.05	2	1	0	0	0	0
Oc tr	25	9	1	0.24	17.19	3.85	1	1	0	0	0	0
Ps ci	15	7	0	0	0	0	18	6	1	0.34	19.65	4.51
Ps co	208	20	3	0.41	3.97	1.48	206	20	4	0.67	4.32	1.88
Ps cy	95	13	0	0	0	0	61	10	2	0.64	9.73	3.13
Ps di	11	3	0	0	0	0	21	6	0	0	0	0
Ps fe/ho/lo	119	21	2	0.31	5.09	1.62	26	17	3	3.2	27.04	11.32
Ps si	2	2	0	0	0	0	0	0	0	0	0	0
Total	2618	298	25	0.64	1.4	.97	1748	227	33	1.9	3.44	2.59

Species key: Ae al- *Ae albopictus,* Ae.ep- *Aedes epactius,* Ae. tr- *Aedes trivatattus,* Ae. ve- *Aedes vexans,* An. pu- *Anopheles puntipennis,* An. qu- *Anopheles quadrimaculatus,* Cq. pe- *Coquillettidia perturbans,* Cs. in- *Culiseta inornata,* Cx. pe- *Culex perturbans,* Cx. qu/re- *Culex quinquefasciatus/restuans,* Cx. sa- *Culex salinarius,* Cx. ta- *Culex tarsalis,* Oc. at- *Ochleratatus atlanticus,* Ps. ci- *Psorophora ciliata,* Ps. co- *Psorophora columbiae,* Ps. cy- *Psorophora cyanescens,* Ps. di- *Psorphora discolor*, Ps. fe/ho/lo- *Psorophora ferox/horrid/longipalpus,* Ps. si- *Psorophora signipinnis*

Table 4.3 Total number of mosquitoes collected at each of the rural trapping sites and the maximum likelihood of infection at each site and overall for all rural sites.

Site	# individuals rural	# pools rural	# pos pools rural	Infection rate
1	229	26	0	0
2	269	24	0	0
3	230	19	2	0.88
4	69	16	0	0
5	187	29	0	0
6	99	23	3	0.53
7	110	16	2	1.73
8	263	26	1	0.43
9	317	27	5	1.29
10	210	17	6	2.08
11	93	11	3	1.88
12	80	14	2	2.43
13	103	13	1	1.01
14	109	14	0	0
15	66	12	0	0
16	184	19	0	0
Total	2618	298	25	0.97

Table 4.4 Total number of mosquitoes collected at each of the urban trapping sites and the maximum likelihood of infection at each site and overall for all urban sites.

Site	# individuals urban	# pools urban	# pos pools urban	Infection rate
17	31	11	6	16.59
18	439	13	1	0.79
19	71	11	9	22.45
20	226	27	9	5.08
21	30	12	0	0
22	57	14	5	9.3
23	196	25	0	0
24	18	8	0	0
25	30	7	0	0
26	137	14	0	0
27	60	10	0	0
28	160	21	0	0
29	32	9	0	0
30	27	9	3	17.02
31	61	13	0	0
32	173	23	0	0
Total	1748	227	33	2.59

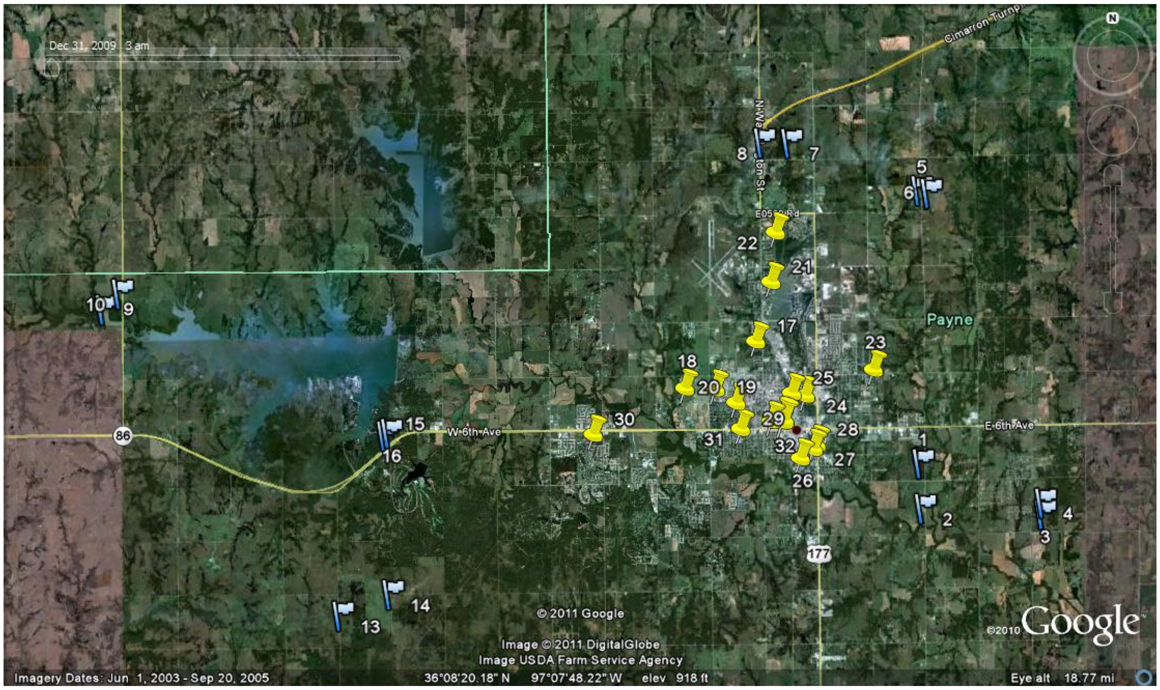

Figure4.1 Blue flags represent rural sites (1-16) and yellow pushpins (17-32) represent urban sites. There are eight rural sites east of Stillwater and eight rural sites west of Stillwater. Each site's resting boxes were sampled weekly. Carbon dioxide traps were used three times during the season. Map taken from Google Earth, March 15 2011 (Google Inc., Menlo Park, CA).

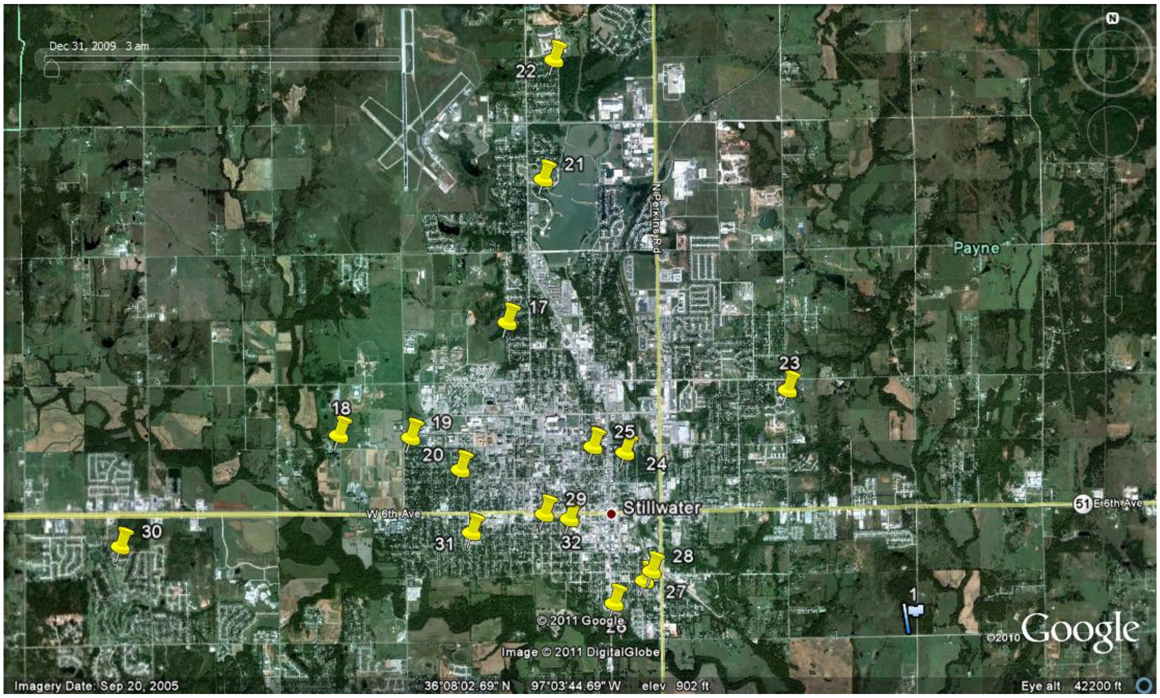

Figure 4.2. Close-up view of urban sites located throughout Stillwater. Sixteen urban sites were located throughout Stillwater. Map taken from Google Earth, March 15 2011 (Google Inc., Menlo Park, CA).

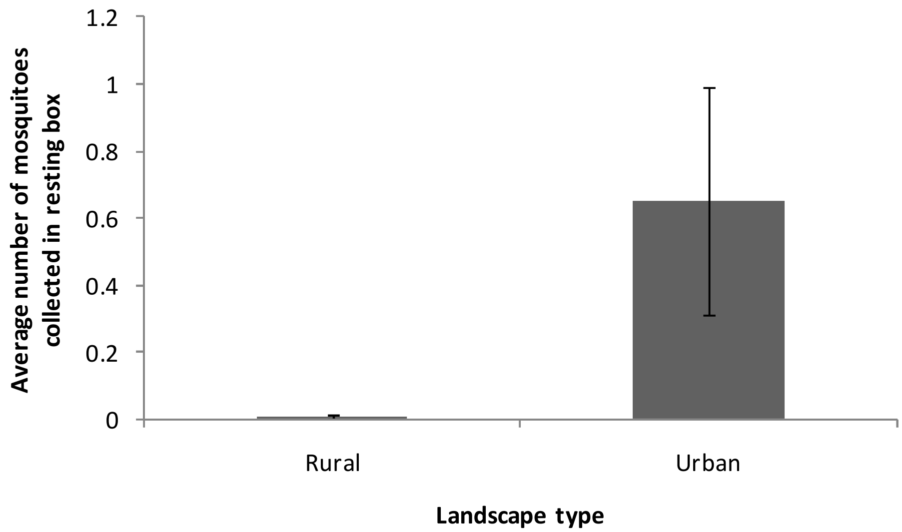

Figure 4.3 The average number of mosquitoes collected per trap day in all rural and urban locations. Significantly more mosquitoes were collected in urban resting boxes than rural resting boxes. (Mann Whitney test: $U_{(16,16)} = 221.5$, $P = 0.0286$). Error bars are +/- 1 SEM.

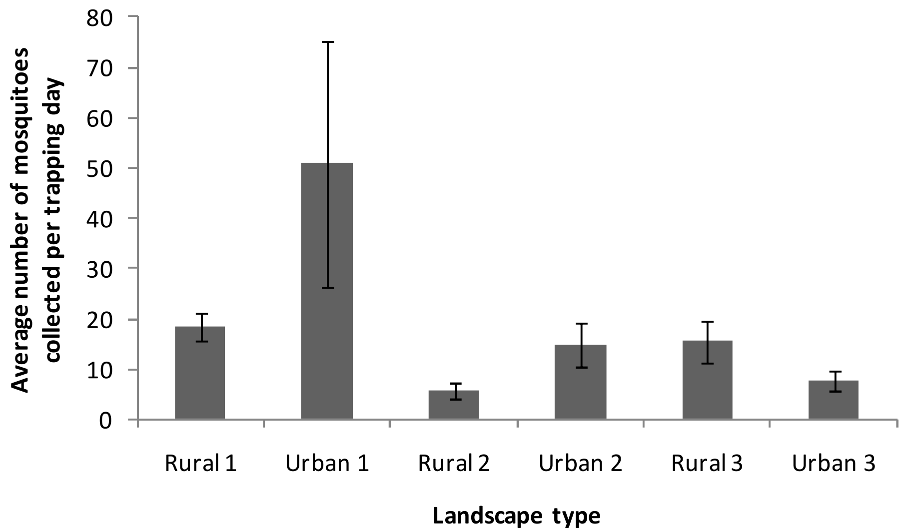

Figure 4.4. Average number of mosquitoes collected in carbon dioxide traps on three trap dates, July 13, August 17, and August 24, 2010. There were no significant differences based on landscape type for any trap dates (July 13: Mann Whitney test $U_{(16,16)} = 249$ $P= 0.5842$, August 17: $U_{(16,16)} = 218.5$ $P= 0.0887$, August 24: $U_{(16,16)} =202$ $P= 0.5079$). Error bars are +/- 1 SEM.

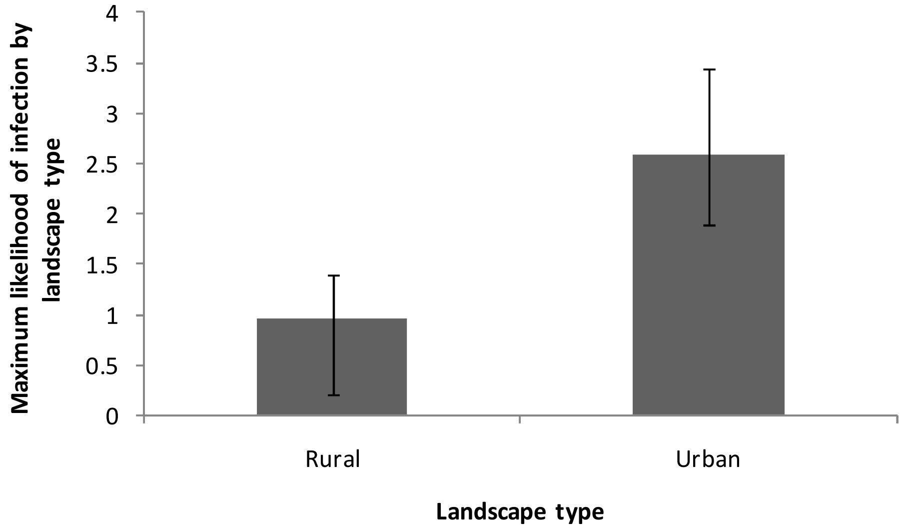

Figure 4.5. Maximum likelihood of infection by landscape type. Significantly more mosquitoes were infected with dog heartworm in the urban than rural landscape. Urban sites MIR 2.59% (95% CI 1.9-3.44%) Rural sites MIR 0.97% (95% CI 0.64-1.4%). Error bars are 95% CI.

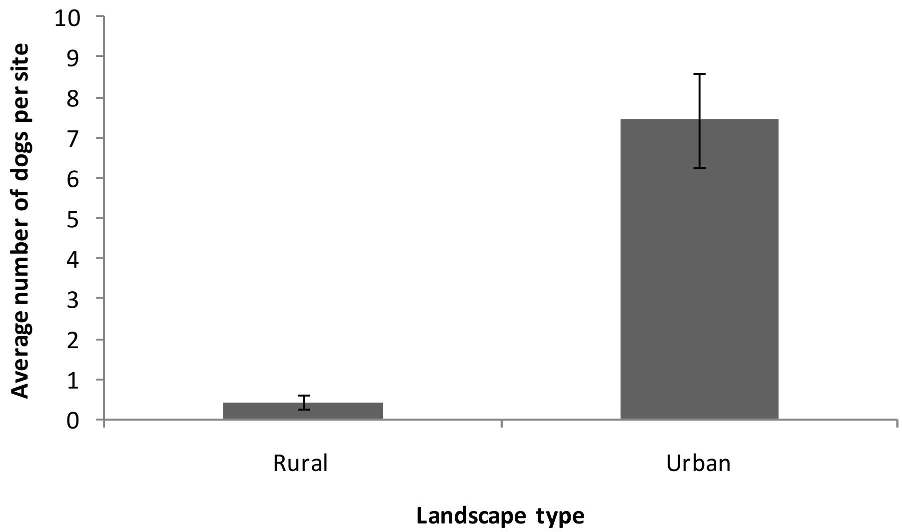

Figure 4.6 Average number of dogs counted in the urban and rural landscapes. Significantly more dogs were observed in urban than rural landscapes t-test t= -6.04 (P< 0.0001). Error bars are +/- 1 SEM.

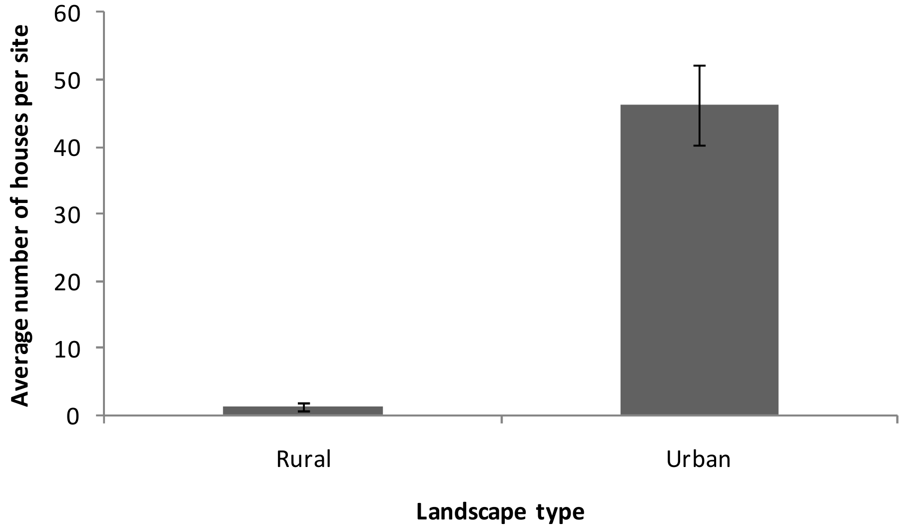

Figure 4.7 Average number of houses counted in urban and rural landscapes. Significantly more houses were observed in urban than rural landscapes t-test t= -7.52 (P<0.0001). Error bars are +/- 1 SEM.

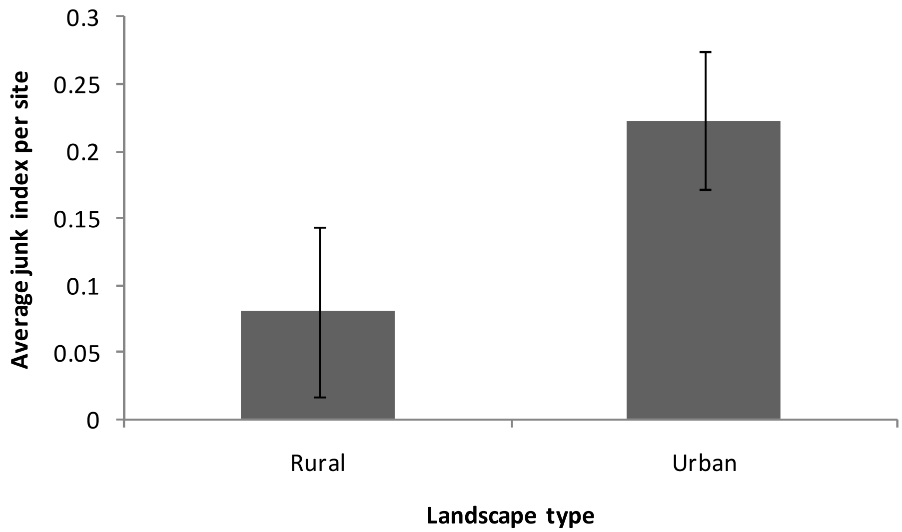

Figure 4.8. Average junk index rating in urban and rural landscapes. The urban landscape had a trend towards higher rating than the rural sites (t-test, t= -1.72, P=0.0953). Error bars are +/- 1 SEM.

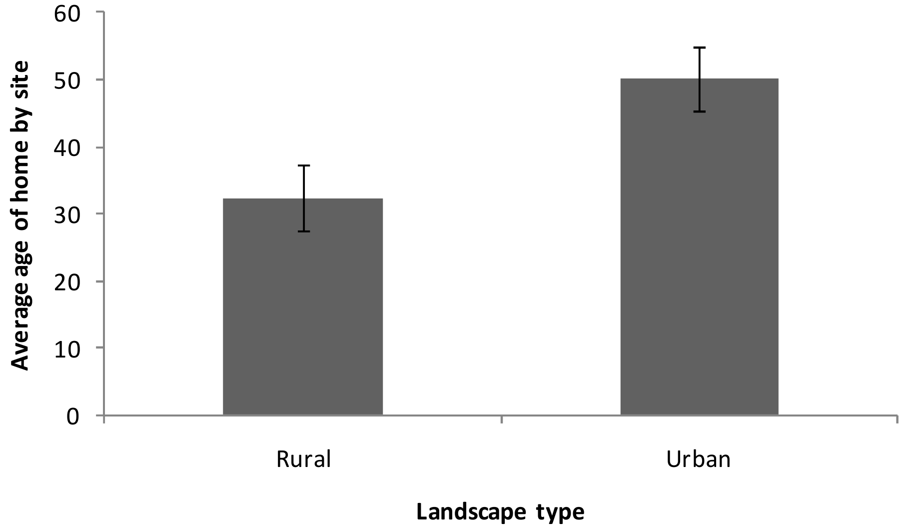

Figure 4.9 Average age of home in urban and rural landscapes. The urban homes were significantly older than the rural homes (t-test, t= -2.11, (P=0.0486). Error bars are +/- 1 SEM.

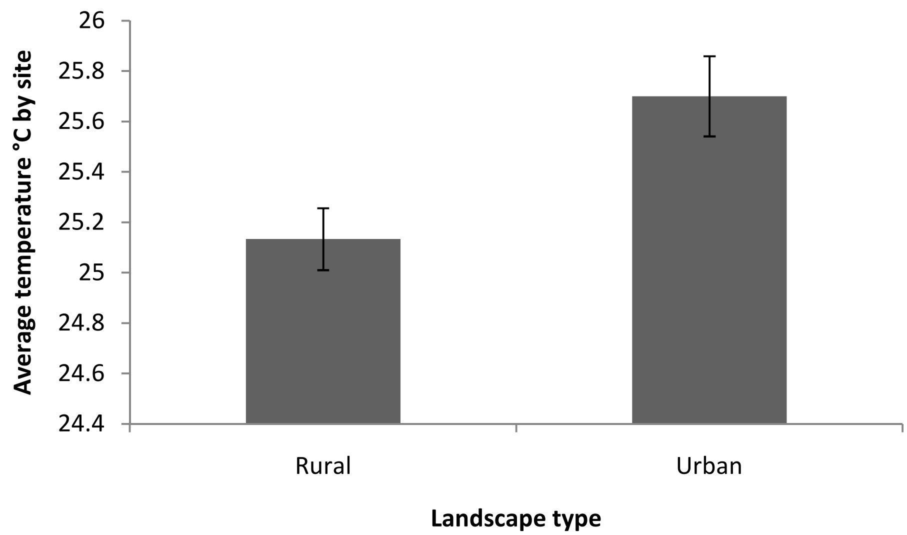

Figure 4.10. Average temperature in July, 2010 in urban and rural locations. The urban average temperature was significantly warmer than the rural average temperature(t-test, t= -2.90 (*P*=0.0073). Error bars are +/- 1 SEM.

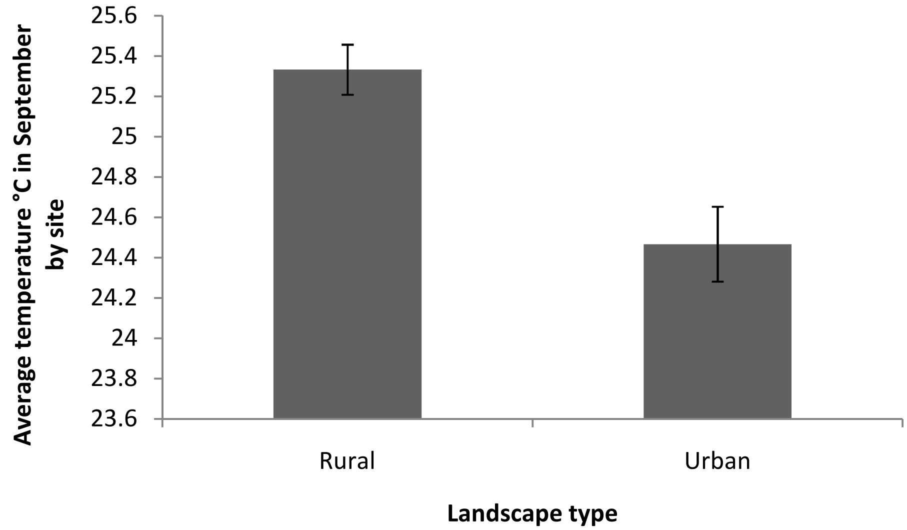

Figure 4.11. Average temperature in September, 2010 in urban and rural locations. The rural temperature was significantly warmer than the urban average temperature (t-test, t= 3.92 (P=0.0005). Error bars are +/- 1 SEM.

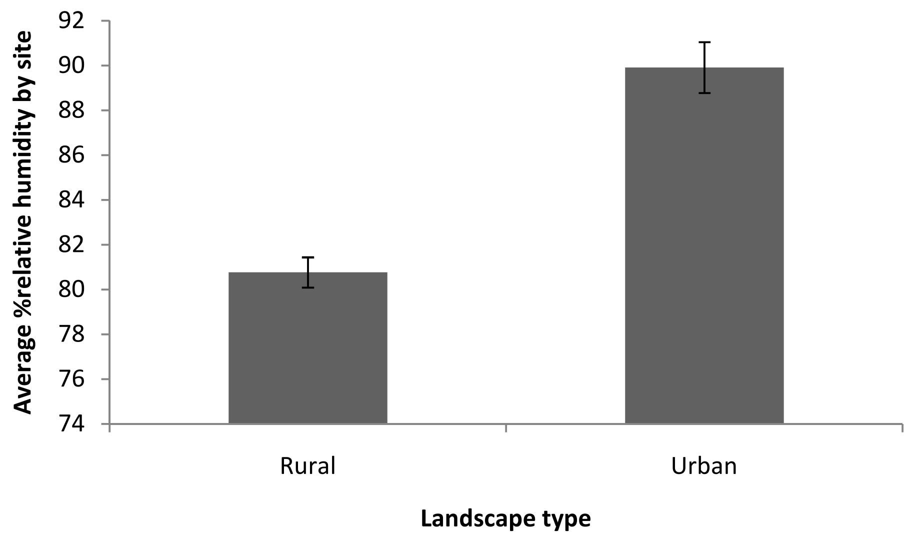

Figure 4.12 Average humidity in urban and rural locations in September, 2010. The humidity in urban locations was significantly higher than in rural locations (t-test, t= -6.93 (P<0.0001). Error bars are +/- 1 SEM.

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CHAPTER V

SUMMARY AND CONCLUSIONS

Dog heartworm was found in both the vector and the host in Oklahoma. Heartworm does not appear to be a significant disease in rural coyotes in Oklahoma and north Texas. We found a 6.49% prevalence rate. This low prevalence of heartworm infections in rural areas is consistent with the lower prevalence of heartworm in rural mosquitoes. In urban areas, mosquitoes had a 2.59% maximum likelihood of infection which was significantly higher than the 0.97% maximum likelihood of infection that was found in rural areas of Payne County.

There is a large range of prevalence rates reported in the literature. On the Pacific Coast in Washington and some areas of California, no heartworm was detected (Foreyt 2008, Sakcs et al 2004). However, along the Gulf Coast in Louisiana and Texas, a 71% prevalence rate was found (Custer and Pence 1981). The Oklahoma coyotes fit in at the lower end of the spectrum perhaps due to concentration of suitable hosts for *D. immitis*, vector species composition, suitability of rural cattle ranches for vectors to inhabit, or variation in the landscape.

The mosquito data collected in Payne County revealed that there is a higher likelihood of heartworm infected mosquitoes in urban areas than rural areas. *Aedes albopictus* and *Psorophora columbiae* appear to be the important vectors of heartworm in urban areas. *Psorophora columbiae* is also an important vector in rural areas of Payne County. As with the coyote data

This could be attributed to the landscape data, number of suitable hosts, vector species composition and vector biology. In urban locations there are more anthropogenic pieces of garbage that can be utilized by container breeding mosquitoes such as *Ae. albopictus*. There are also more dogs in urban areas and if untreated with prophylactic anthelmenthics, will increase the number of suitable hosts for *D. immitis.* More *Ae. albopictus*, which is an important vector species, were trapped in urban areas indicating that the species thrives more in urban landscape type than a rural landscape type in Payne County, Oklahoma.

The implication for this study are that dogs living in Payne County, especially urban areas of Payne County are at risk for heartworm infection. They should be given preventative treatment regularly to reduce the chance for infection with this deadly disease.

Due to the low prevalence rate of heartworm infections in rural Oklahoma coyotes, future studies should look at the prevalence of heartworm in coyotes living in Tulsa or Oklahoma City. Based on studies conducted in Illinois, coyotes from rural areas of the state had a 4.6% prevalence rate, while those in Chicago had a 41% prevalence rate (Nelson et al. 2003, Gehrt unpublished data). Researchers in Oklahoma could also do a concentrated study about the infections rates found in domestic dogs and cats brought to animal shelters. Payne County is not a highly urbanized area, so a mosquito study in a larger urban center could also be conducted to determine if the prevalence of heartworm in mosquitoes continues to increase as the size of the city increases.

VITA

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Candidate for the Degree of

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Title of Study: EPIZOOTIOLOGY OF DOG HEARTWORM, *DIROFILARIA IMMITIS*, IN OKLAHOMA

Pages in Study: 76 Candidate for the Degree of Master of Science

Major Field: Entomology and Plant Pathology

Scope and Method of Study:
There were four main objectives of this study. The first was to determine the prevalence of *D. immitis* in coyotes throughout Oklahoma. The second was to evaluate the relationship between landscape and social factors and the number and species of heartworm positive mosquitoes. The third was to determine which species of mosquitoes trapped are infected with *D. immitis* in Payne County. The final objective was to determine the importance of *Ae. albopictus* in *D. immitis* infections in Payne County.

These objectives were accomplished through a coyote study and a mosquito study. In an effort to learn more about the sylvatic cycle of heartworm in Oklahoma, whole blood and serum samples were collected from 77 coyotes on ranches and in rural areas in seven counties throughout the state from January to March, 2010. Coyote carcasses were donated by the Oklahoma Department of Agriculture, Food, and Forestry USDA Wildlife Services and the Oklahoma Predator Hunters' Association .Blood was collected from harvested coyotes and tested for heartworm using SNAP antigen tests. Mosquitoes were collected using three trapping methods, resting boxes, carbon dioxide traps, and BG sentinel traps, from 16 rural and 16 urban locations in Payne County. They were tested for heartworm using RT-PCR.

Findings and Conclusions:
There is a 6.49% prevalence rate of *D. immitis* in rural Oklahoma coyotes. Urban mosquitoes have significantly higher rates of *D. immitis* than rural mosquitoes. *Aedes albopictus* and *Psorophora columbiae* are important vectors of *D. immitis* in Payne County, Oklahoma. Urban collected mosquitoes had significantly higher maximum likelihood of infection, 2.59%, than rural collected mosquitoes, 0.97% (P<0.05). Two species, *Aedes albopictus* and *Psorophora columbiae* emerged as important vectors if heartworm in Payne County.

ADVISER'S APPROVAL: Michael Reiskind