

VECTOR-BORNE DISEASES OF SAN ANGELO, TEXAS

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MATTIE RITA PRICE

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VECTOR-BORNE DISEASES OF SAN ANGELO, TEXAS

by

MATTIE RITA PRICE

APPROVED:

Dr. Nicholas Negovetich, Chair
Assistant Professor of Biology

Dr. Laurel Fohn
Assistant Professor of Biology

April 28, 2017
Date Successfully Defended and
Approved by Advisory Committee

APPROVED:

Dr. Shirley M. Eoff
Director of the Honors Program

May 12, 2017

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ABSTRACT

Mosquitoes are vectors of viral and parasitic diseases. Some diseases carried by mosquitoes are encephalitis, filariasis, dengue, yellow fever, and malaria. The goal of this research is to identify mosquitoes in San Angelo, Texas, and the human and domesticated animal diseases reported to be vectored by these species. Therefore, in late Fall 2016, mosquitoes were collected from San Angelo using a commercially available CO₂ trap. Identified species include *Anopheles pseudopunctipennis*, *An. judithae*, *An. punctipennis*, *An. crucians*, *An. perplexens*, *An. barbei*, *An. atropos*, *Psorophora columbiae*, *Ochlerotatus hendersoni*, *Mansonia titillans*, and *Culex erraticus*. Of these, the most common species were *C. erraticus* and *An. pseudopunctipennis*. This information may educate people to be aware of these potentially harmful organisms, realize that these common “pests” are more dangerous than they appear, and influence individuals to take safety measures to reduce the chance of acquiring vector-borne diseases.

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INTRODUCTION

Mosquitoes serve as vectors of parasitic and infectious diseases, meaning they may transmit diseases from host to host and perpetuate the disease process via completing the pathogen's life cycle. There are two major forms of mosquito-borne diseases, viral and parasitic, distinguished by the group of organisms that cause the disease—viruses or parasites. This division will serve to separate the diseases investigated in this study. The viral category includes West Nile virus, Zika virus, chikungunya fever, dengue fever, yellow fever, St. Louis encephalitis, California encephalitis, Western equine encephalitis, Eastern equine encephalitis, and Venezuelan equine encephalitis. On the other hand, malaria and filariasis fall under the parasitic category. These diseases are addressed in detail in the following paragraphs.

There are approximately 85 different species of mosquitoes in Texas.¹ These species transmit a variety of pathogens to humans causing significant disease including West Nile virus, Zika virus, chikungunya fever, dengue fever, Yellow fever, St. Louis encephalitis, California encephalitis, Western equine encephalitis, Eastern equine encephalitis, Venezuelan equine encephalitis, malaria, and filariasis. Of the 85 mosquito species found in Texas, 20 of them can be found in Tom Green County and most are reported as vectors for a variety of viral and parasitic diseases (Table 1).¹ Should an outbreak occur, these 20 species may be important vectors that can maintain a disease in this area.

Table 1. The 20 mosquito species reported in Tom Green County.¹

Mosquito species	Diseases reported to be vectored
<i>Aedes nigromaculis</i>	None reported
<i>Aedes sollicitans</i>	Eastern equine encephalitis ² ; West Nile virus ³
<i>Aedes vexans</i>	Eastern equine encephalitis ² ; West Nile virus ³
<i>Anopheles pseudopunctipennis</i>	Malaria ⁴
<i>Anopheles punctipennis</i>	Dog heartworm ⁵ ; malaria ⁴ ; West Nile virus ⁶
<i>Culex erraticus</i>	Eastern equine encephalitis ² ; St. Louis encephalitis ² ; West Nile virus ⁶
<i>Culex (Melanoconion) sp.</i>	Venezuelan equine encephalitis ⁷
<i>Culex quinquefasciatus</i>	Western equine encephalitis ² ; St. Louis encephalitis ² ; West Nile virus ² ; Dog heartworm ²
<i>Culex restuans</i>	West Nile virus ²
<i>Culex salinarius</i>	West Nile virus ²
<i>Culex stigmatosoma</i>	West Nile virus ³
<i>Culex tarsalis</i>	St. Louis encephalitis ² ; Western equine encephalitis ² ; West Nile virus ²
<i>Culex thriambus</i>	None reported
<i>Culiseta inornata</i>	Western equine encephalitis ² ; West Nile virus ²
<i>Psorophora ciliata</i>	None reported
<i>Psorophora columbiae</i>	Dog heartworm ⁵ ; West Nile virus ⁶
<i>Psorophora cyanescens</i>	None reported
<i>Psorophora discolor</i>	None reported
<i>Psorophora signipennis</i>	None reported
<i>Uranotaenia syntheta</i>	None reported

Viruses and blood-borne pathogens can be transmitted by a variety of organisms. For example, arboviruses are transmitted via arthropods, which take blood meals on vertebrates. The cycle they are involved in is complex, and a small number of these arboviruses can cause health issues for humans.⁸ Multiple routes of transmission between the vertebrate hosts are considered possible. For example, transmission via the hematophagous vector and blood transfusions are known to be a possible way for viruses to enter human hosts leading to disease.⁹ Long distance movement of both the host or vector also help spread disease; this could occur by humans traveling abroad to endemic areas, becoming infected in this area, and bringing back this disease once returning home. If the correct vector is present in the patient's home city, then there could be an outbreak domestically.¹⁰ One could then hypothesize that the vectors could regionally move as a result of climate analogous to what many other species are commonly known to do seasonally, or be transported accidentally via mass transportation like on an airplane or cargo ship. Zoonotic transmission is an additional transmission route.¹¹ It can be postulated that individuals entering natural habitats of organisms harboring these pathogens or some exposure to these organisms without entering their homes can present a situation in which humans can become infected. Sexual transmission, along with vertical transmission, are additional ways these viruses can be spread from person-to-person or from mother-to-child.^{11,12} It can be imagined that the transmission from mother-to-child can occur in utero through the placental wall, congenitally, while breast-feeding from the mother, or another form of direct contact between

fluids. In the following paragraphs pathogens affecting humans in Texas and more specifically the city of San Angelo will be discussed in further detail.

The West Nile virus is a pathogen and a flavivirus, therefore of the *Flaviviridae* family, that can cause encephalitis in this area and has been ubiquitously seen in the news for infecting humans.^{13,14,3} Local news coverage of this disease showed four cases leading to one fatality in 2012 in San Angelo, Texas.¹⁵ The physician questioned, Dr. Irv Zeitler, Vice President of Medical Operations at Shannon Hospital, remarked that infection by this virus could be based on exposure to mosquitoes alone without any other substantial risk factors. Dr. Zeitler also noted that supportive treatments are the best way to treat the disease and that the sole way to “cure” this disease is by way of one’s own immune system fighting off the virus.¹⁵ This may startle many because there is no quick and easy way to instant rejuvenation. Of greater concern may be the statistics provided by Christine Mann, who is a spokeswoman for the Texas Department of State Health Services. According to her, in 2012 there had been “16 deaths and more than 380 cases...confirmed around the state, many of them in the Dallas-Fort Worth area, where nine deaths have been reported.”¹⁵ This information shows how serious this disease can be in areas with favorable environmental conditions for the mosquito life cycle. West Nile virus, along with other mosquito-borne illnesses, can incur many costs not only to the individual’s health, as seen with the above example, but also to the healthcare system and the state. For example, in Louisiana from mid-2002 until early 2003, West Nile virus cases cost \$20.1 million—\$10.9 million related to the infection, with \$4.4 million of this related to medical costs and \$6.5 million related to non-medical costs,

and \$9.2 million involving the public health response.¹⁶ This information serves as a reminder that these mosquitoes are a cost to the population's health and the local and national economy.

The West Nile virus causes much concern not only because of its fairly untreatable nature, but also because of its pathology. Birds act as the primary host for this virus and exposure via handling alive or dead birds contributes to the risk of acquiring infection.³ This virus was first discovered in Uganda and entered the state of Texas in 2002. As of 2014, the Texas Department of Health Services reported 2,513 total cases in Texas, including four cases in Tom Green County.¹⁷ This virus causes many symptoms, ranging from non-specific symptoms to more severe symptoms, including encephalitis. Encephalitis is inflammation of the brain, most often resulting from a viral infection.¹⁸ The various symptoms depend on the strain of West Nile virus but include mental changes, gastrointestinal complaints, severe muscle weakness, fever, headache, and other cold or flu-like symptoms. Immunosuppression and underlying disease processes are frequently associated with this viral disease, namely diabetes mellitus, coronary artery disease, and hypertension. Increased age is also a risk factor for West Nile virus.³

Humans are not the only organism affected by West Nile virus. Domesticated horses are also susceptible. In 2014, Texas reported 25 cases of equine West Nile virus.¹⁷ The infection can result in symptoms leading to an asymptomatic presentation or even death via encephalitis. Compared to humans, infected horses develop encephalitis at a greater rate.³ The changes experienced by these animals after infection include neurological issues such as

paralysis, and other problems associated with mood and increased desire to sleep.³ Forty percent of infected horses that survive and recover from the virus show signs of neurological damage several months afterwards. This virus shows no preference for horses of a specific age. Thus, all age groups are at risk. Clinical signs are said to present themselves in 10-39% of those infected and include neurological symptoms such as issues with body movement. Unlike for humans, a vaccine for horses does exist and is the best way to protect domestic horses from these harmful symptoms.¹⁹

The Zika virus, also a flavivirus, has become a prominent story in the media because of the pathology that presents with this infection and the rapid spread of this virus via sexual contact and the mosquito vector.^{20,8} Another reason for its fame is its publicity in the 2016 Summer Olympics in Rio de Janeiro, Brazil. The fact that a great number of travelers would be entering this area of Brazil, an area known to have the disease and the correct species of mosquito to vector the disease, was of great concern. Also, the seemingly asymptomatic nature of this virus worried many.²¹ Symptoms of this virus that occur in most infected individuals include a rash, fever, and headache. These are innocuous pathologic effects, but what makes this disease deserving of special attention is the significant morbidity exhibited by unborn children. The virus causes microcephaly to occur in the fetuses of pregnant women leading to cognitive damage and even death. There are no known vaccines for this virus, and the lack of significant pathology in infected adults is a serious concern in areas in which this virus is present in the population, especially for the pregnant or soon-to-be pregnant women.²² This concern has been recently expressed to the citizens of Texas. For

example, as of early April 2017, a news release recommended that Texas residents in the Rio Grande Valley be tested for the Zika virus if pregnant or exhibiting any signs or symptoms of this disease.²³ This news report also identified rising temperature increase with the onset of summer as contributing to elevated numbers of mosquitoes present and therefore a greater risk of becoming infected with this virus.²³ This concern is valid because in August of 2016 Texas's first infant death was reported as a result of microcephaly. The mother had acquired the infection while traveling. The virus then crossed the placental barrier and infected the fetus.²⁴ This particular news release also noted that a month earlier in July 2016, a different child was reported as the first infant in Texas to develop microcephaly as a result of this disease.²⁴

According to the World Health Organization and the New England Journal of Medicine, the Zika virus was discovered in Uganda.^{25,8} This virus's main hosts were primates and the *Aedes africanus* mosquito. From 2014 to 2015, a 20-fold increase in Zika virus cases was observed in Brazil, causing health officials to declare that a Zika virus outbreak had occurred.^{8,25} This recent escalation in Zika virus cases led to the great publicity in the Americas, including the United States. As of March 2016, the United States had seven confirmed cases of Zika virus infections.²⁶ This number rapidly increased to over 5000 symptomatic cases of Zika virus reported in the United States in all of 2016.²⁰ The vast majority of these cases were a result of individuals traveling from endemic areas of this virus; the second most amount of cases stemmed from local mosquito infection notably in Florida and Texas; and, the fewest number of cases were a consequence of sexually

transmitted infections.²⁰ Zika virus infections have been reported in Texas. For example, a case of sexually-transmitted Zika virus was reported in Dallas county, Texas, in 2016. The patient's sexual partner had acquired this virus while abroad and infected a Dallas county resident. This case is considered the first domestically-acquired infection.²⁷ From January to May 2017, 110 cases have thus far been reported solely from the traveling route.²⁸ Not all cases are related to travel. Specifically, local transmission of this disease has likely occurred within the last year around Brownsville, Texas.²⁹ The Zika virus is naturally vectored by mosquitoes, specifically members of the *Aedes* genus, but it has also been shown that transfer of this disease could occur perinatally and through blood transfusions. Sexual intercourse is an additional route of transfer of this virus between humans.³⁰

The Rio Olympics of 2016 also serves as a great example of what could lead to rapid spread of the Zika virus because of our global, interconnected nature. Concern developed over the great number of visitors to Rio, and therefore possible infection of these individuals, who would return to their home countries at the conclusion of the Olympic Games.²¹ With the virus living inside these individuals, the potential for transmission is high. Transmission via sexual contact can cause rapid spread in the human population thus increasing the likelihood of entry into the mosquitoes.²⁰ Once in the mosquitoes, the virus can remain in the area and maintain a significant risk of infection in the human population.

Dengue and chikungunya fever are two more viruses transmitted via mosquitoes. Chikungunya is an alphavirus (family *Togaviridae*), while dengue is a flavivirus.^{8,31} The species of mosquitoes responsible for the infection of people with the viruses causing these

diseases are *Aedes aegypti* and *Aedes albopictus*.³² Both of these species are present in other areas in the state of Texas.¹ These two diseases are said to cause symptoms of a short nature but can in some cases debilitate individuals for a period of time, sometimes as long as multiple years as seen with chikungunya fever. These less commonly-experienced, long-lasting symptoms include peripheral joint pain. According to the Texas Department of State Health Services, before 2013 fewer than five cases of chikungunya fever were reported in the state, while as of 2014, 114 cases of this disease were reported in the state.¹⁷ These cases involved patients who traveled to Texas from endemic areas.¹⁷ Both dengue and chikungunya fevers present as seemingly asymptomatic infections with a fever, rash, headache, and body pains. Dengue fever presents hemorrhagic complications for a select number of those individuals infected.³² Some variation in presentation of symptoms exist when comparing the age of those individuals infected with the dengue virus. For example, adults tend to experience organ involvement and hemorrhaging as a part of their pathological process. Children, on the other hand, are more likely to experience vascular leakage and shock.³³ The following attention-catching statement concerning the viral disease dengue fever illustrates the potential of this disease if given the optimal conditions: “Dengue is the world’s most common mosquito-borne viral disease (WHO, 2012a).”³² The World Health Organization (WHO) is understood to have some of the most up-to-date information on diseases and health related issues across the globe, therefore providing a reliable and current source of information on infectious diseases. This statement about dengue’s common status may come as a surprise because of the lack of wide publicity of this mosquito-borne disease compared

to others like the West Nile and Zika viruses. This may be because of the general lack of severe symptoms that would generate sensational news headlines and the tendency of the United States' media, and media in general, to only report on stories of greater excitement. According to the Texas Department of State Health Services, between 2003 and 2013, a total of 249 cases of dengue fever had been reported in Texas, and in 2014, 34 cases of this disease were reported with all cases involving travelers.¹⁷ With increased travel between countries, the dengue virus and chikungunya virus have the potential to infect individuals who normally would not have come in contact with these diseases. For example, there have been cases of both of these mosquito-borne diseases in the United States as a result of travel from areas like the Caribbean.³² Dengue has been increasing in its presence across the globe recently as a result of anthropogenic factors. Increasing urbanization that leads to more mosquito breeding sites near residential areas, increases in international travel, and the great growth of the global population have proven to aid in dengue's rising incidence.³³ Also, with changes in distribution of mosquitoes over time, who is to say that these vectors won't be inclined to shift regions in the state and the country? Although the mosquito species that vector the dengue and chikungunya viruses are present in Texas at this time, they have not been reported in Tom Green County. However, northern and western migration of the mosquito vectors allows one to infer that these diseases have the possibility to expand their range, thereby infecting more people.¹

Yellow fever is a flavivirus that has a substantial history in the United States and proved to be an unexpected challenge to combat.³⁴ The discovery of *A. aegypti* as the vector

led to successful efforts of proper sanitation, decontamination, and mosquito containment measures resulting in ridding the United States of this disease. As a result of these labors, much was learned about this jaundice-inducing disease that helped to greatly advance some of the medical facilities still in operation today. For example, the University of Texas Medical Branch in Galveston advanced greatly following the institution's experience with this disease.³⁵ Yellow fever is often accompanied by noticeable symptoms such as hemorrhaging, sometimes a jaundicing of the skin, and most notably black vomit.³⁶ The pathology occurs in three stages: infection, remission, and intoxication. The infection begins with flu-like symptoms, accompanied by slow heart rate, fever, and reduced white blood cell count. During remission, it is common for patients to recover, but those 15-20% of patients who do not recover advance to the intoxication stage. Symptoms such as hemorrhaging, fever, jaundice, liver malfunction, issues with organ systems including the kidneys, heart, and central nervous system, and shock may occur. These symptoms cycle every 24-48 hours. Unfortunately, 20-60% of severe cases result in fatality.³⁷

Several historical events in the United States are linked to yellow fever. Memphis, Tennessee, had a rough time with this disease leading to a substantial decrease in population size in the 1870s. This decimation led to many events and changes in this city, including regaining control from the clutches of yellow fever after extensive efforts to save their citizens from this ravaging virus. Also, a greater involvement of the black community in services such as the police force occurred as a result of the revitalization of this metropolitan area. This integration had not been seen in the United States prior to this time.³⁶ Another

historically significant event associated with this disease is the eradication of yellow fever for construction of the Panama Canal at the turn of the 20th century. The environment of Panama is one that is favorable for mosquito life. The amount of rainfall, lack of drainage, high temperatures, and much more led to the thriving of yellow fever, and other mosquito-borne diseases such as malaria, that plagued the workers constructing the Panama Canal.³⁸

President Theodore Roosevelt allocated much money and resources in order to alleviate the risk of disease so that the Panama Canal could be completed. Research revealed the importance of the mosquito-virus interaction, showing that eliminating or reducing mosquito populations can limit yellow fever transmission to the human population. Following the successful efforts in Panama, mosquito control measures were then implemented in the United States and resulted in many changes that improved the overall health of the U.S. populace.³⁹ For example, significant sanitation improvements in the south not only gave way to eradication of the yellow fever virus, but also provided an environment of improved overall health in the population and prevention of other harmful diseases.³⁶

Recent cases in the Americas indicate this virus is still a danger to certain populations. In early 2017 in Brazil, rural outbreaks of a strain of yellow fever virus occurred. These cases are referred to as “sylvatic” or “jungle” cases because humans were accidentally infected by a virus that naturally infects non-human primates living in the forest. This current outbreak has resulted in 234 infections with 80 fatalities. A major concern of this outbreak revolves around infected individuals traveling and therefore possibly infecting more people. There is little concern with infections taking hold in the interior of the United

States as a result of the low mosquito density and limited exposure to these vectors. But, there is much greater concern for the Gulf Coast region where mosquitoes are more common and abundant. A vaccine does exist that, according to the World Health Organization, provides life-long protection for 99% of individuals vaccinated. Actions like administering vaccinations and proactive preventative measures are imperative to control and reduce the number of yellow fever cases across the globe.³⁷

Equine encephalitides and St. Louis encephalitis are additional mosquito-borne viral diseases that are found in the state of Texas, and specifically in Tom Green County (Tables 1 and 2). California encephalitis is also grouped with these diseases and therefore will be discussed. Western equine encephalitis, Eastern equine encephalitis, and Venezuelan equine encephalitis all are alphaviruses.³¹ St. Louis encephalitis is a flavivirus, and the California encephalitis are bunyaviruses (family *Bunyaviridae*).⁴⁰ Venezuelan and Western equine encephalitis viruses cause disease in the central nervous systems of both horses and humans.⁴¹ Therefore, the Western equine encephalitis presents with symptoms commonly seen with viral infections such as headaches, as well as other symptoms of a nervous system nature such as seizures and altered consciousness. Additionally, this particular virus is found in the midwestern and western regions of the United States, affecting very young individuals and those past the age of 50. This virus results in death of 5-15% of infected individuals.⁴⁰ Eastern equine encephalitis is found in the United States, specifically along the Gulf and East Coast regions. This mosquito-borne disease has a high morbidity and mortality, with about one-third of infected individuals dying. The symptoms of this virus are similar to all other

viral infections and include abdominal pain, headache, and fever. After some time severe encephalitis takes hold and nervous system destruction accompanied by seizures occurs.⁴² St. Louis encephalitis is another viral mosquito-borne disease which occurs in the United States. This virus exhibits year-round transmission in Texas.¹⁷ Investigation into the origin of the virus in the United States revealed the virus likely came from South or Central America. Preliminary research suggests two possible modes of movement into the country. The virus could have been introduced by a traveling human who was infected via mosquitoes in an endemic area.⁴³ Alternatively, introduction could have occurred when infected migratory birds traveled through the United States and transmitted the virus to mosquitoes.⁴³ Lastly, the California serogroup viruses are a category of viral mosquito-borne infections that are widespread across the United States. This group includes the California encephalitis virus. This virus leads to the common symptoms of viral infections, such as fever and headache. About 56% of patients with this infection show signs of meningeal irritation, and 49% present with seizures.⁴⁴ All of these viral diseases infect multiple species of mosquitoes (Tables 1 and 2) including *Aedes sollicitans*, *Ae. vexans*, *Culex erraticus*, *C. quinquefasciatus*, *C. tarsalis*, *Culex (Melanoconion) sp.*, *Culiseta inornata*, and *Mansonia titillans*.^{2,7}

The United States has practiced a variety of precautions in order to prevent the further introduction of the Venezuelan equine encephalitis virus into this country. Some of these precautions include limiting equine transport into the country, use of insecticide spray, and vaccination of the equines.⁷ These vaccines, using live and inactivated viruses, are available

for equine use against the Venezuelan equine encephalitis.⁷ Vaccinating humans with the same live virus vaccines as those used in horses results in a plethora of unfavorable reactions including but not limited to fever, headache, myalgia, nausea, and diarrhea, with anecdotal reports of teratogenic effects.^{7,45,46} Therefore vaccination is only recommended for those individuals in possible direct exposure to the virus.⁷ Fortunately, spread of the Venezuelan equine encephalitis virus from human-to-human has not been demonstrated and is unlikely to occur.³¹

The great infectivity of some of these encephalitis viruses, especially Venezuelan equine encephalitis, Eastern equine encephalitis, and Western equine encephalitis, makes them a potential pathogen for use as a bioweapon. These viruses produce significant pathology as previously mentioned. Moreover, the viruses can be mass-produced with ease, are highly infective to humans, can be aerosolized for deployment, and are stable.³¹ The incapacitating component of these diseases occurs in three phases, namely replication of the virus and the eventual spread peripherally through the body, invasion of the nervous system, and degeneration of the neurons in the brain. These three steps often lead to death of the infected organism.³¹

As previously discussed, mosquitoes serve as the vector for numerous viruses which have the potential to impact human health. These are not the only pathogens these arthropods are capable of transmitting. In fact, they may also serve as the vector for parasitic diseases. The following discussion of parasitic mosquito-borne infections illustrates how the symptoms of these two infection types vary and conform to each other.

There are fewer mosquito-borne parasitic diseases of notable influence on human health than diseases caused by viruses. But, those few parasites that cause pathology produce some of the most notorious diseases man has encountered. Malaria, for example, is a disease caused by a protozoan parasite (Phylum Apicomplexa) belonging to the *Plasmodium* genus. Malaria is not normally associated with the United States despite its historical and current presence in the mosquito population. *Plasmodium malariae*, *P. ovale*, *P. falciparum*, *P. vivax*, and *P. knowlesi* are the parasite species known to cause malaria in humans, although each species has some variation of presentation.⁴⁷ During the mid-20th century in the southeastern United States, there was a concentrated effort to rid this country of malaria; however, other countries continue the fight against this debilitating parasite. Around 214 million new malaria cases around the globe were reported in 2015.⁴⁷ While malaria is effectively eliminated from the United States, the presence of the vector could allow the disease to reestablish itself in the population.

The prevalence of travel and immigration from countries still battling malaria increases the potential for infection in areas that harbor the appropriate vector species of mosquito. Notable and commonly known pathology of malaria includes periodic fevers, red blood cell lysis, and a host of other debilitating symptoms. This disease has multiple forms leading to differing presentations and treatment responses. For example, those infected with *P. vivax* may experience flu-like symptoms for about 48 hours followed by the characteristic paroxysm of malaria: (1.) intense feeling of cold with severe shivering and convulsions; (2.) extremely high fever of 104-106°F; (3.) nausea, vomiting, and delirium; and (4.) breaking of

the fever accompanied by profuse sweating.⁴⁸ For *P. vivax*, this cycle lasts 8-10 hours and is repeated every 72 hours. Mosquitoes acquire the parasite by feeding on an infected individual. Once a mosquito is infected, then that mosquito is infected for life and can transmit the parasite to multiple individuals.⁴⁸ Not all humans are infected for a lifetime by malaria. There is potential for a cure if medications are used correctly, for example the right type, dose, and timing. If any of these factors do not align then the parasite could acquire resistance to the medication and harm the patient for life possibly via relapses in symptoms. If an individual is not treated properly or at all, long term damage to their body, for example, kidney failure, coma, confusion, seizures, and even death can occur.⁴⁹ With all of this information in mind one could foresee malaria infected individuals being able to act as a reservoir for an extended period of time. If the infected person traveled, this could pose a threat to the population at any time in their life. If the vector is present to take a blood meal, become infected, and infect others, then it would be possible to have an outbreak of this disease.

Filariasis is a disease caused by filarial, parasitic worms causing lymphedema resulting in limb swelling.⁵⁰ The parasite *Wuchereria bancrofti* (Phylum Nematoda) is responsible for 90% of the burden of this disease. The adult nematodes live in the lymphatic system of the human host and release larval stages, called microfilariae, that travel through the blood and lymphatic system.⁵¹ The symptoms of filariasis are caused by damage to the lymphatic vessels and subsequent blockage of the lymph nodes. Blood-feeding insects, including mosquitoes, acquire the microfilariae during a blood meal. The parasite then

develops to a third stage larvae and is delivered to another human when the vector feeds again.⁵¹ Filariasis is an issue for those living in developing countries and is considered an indicator of poverty.^{51,52} This is because the presence of disease is associated with poor sanitation, lack of clean water, and limited to no vector control.⁵²

Dirofilaria immitis (Phylum Nematoda), also known as dog heartworm, is a filarial nematode that lives in the heart and pulmonary artery of canines.^{53,54} This filarial worm is more likely to have an impact on individuals' lives here in the United States, especially those with dogs as pets, and is most definitely more publicly known compared to other parasites in this category. *Dirofilaria immitis* is transmitted between dogs by mosquitoes. Of the mosquitoes found in this study, *Anopheles crucians*, *An. punctipennis*, and *Psorophora columbiae* are reported to vector the parasite in San Angelo, Texas (Table 2).⁵ Additionally, *Culex quinquefasciatus* is reported in the literature to inhabit Tom Green County and to also vector *D. immitis* (Table 1).²

When considering which animals are capable of being infected by viruses, it is imperative to know how many can serve as hosts for the mosquito-borne diseases mentioned thus far and of present concern for the human population. For example, the Zika virus was first discovered in monkeys, birds act as the primary host for West Nile virus, yellow fever can be hosted by nonhuman primates, and equines are the hosts of the equine encephalitis viruses.^{25,3,37} Given these examples, one could fathom that mosquito-borne diseases that can use hosts other than humans increases the potential danger to the local population. This danger is directly linked to the elusive nature exhibited by some of these diseases.

Specifically, one could infer that if these animals hold the diseases inside them while a part of or near a population of humans, then there could be possible transfer of the disease to the human population via the mosquito vector. This is the presumed route of infection for the jungle cases of yellow fever in Brazil.³⁷ The traveling nature of our species could serve as a spreading mechanism for these diseases to other nations if a proper vector is present. For example, the appropriate mosquito species acting as the vector acquires the virus from the infected human and transmits it to the animal population where it becomes established. No other humans are infected at this time, and because the disease will be linked to travel, the response by public health officials to “contain” the spread of disease will be minimal if any occur. Thus, the virus can be introduced and maintained in the area without infecting a significant number of humans. Once this occurs, the area will now have an increased risk for a significant outbreak in the human population.

After researching mosquito-borne diseases, it is clear that this ever-present pest has much more influence on the past, present, and future lives of those living among them than could have been imagined. With this information one must consider the information presented and all the possible implications this could have on the population in Texas, and more specifically in the city of San Angelo. In order to correlate what is found in the literature to what is found in this west Texas city, this project began as an attempt to answer just how dangerous local mosquitoes could be if given the opportunity to spread infectious diseases. To address this question, the species of mosquitoes that live in this area first had to be identified.

The goal of this project is to identify the mosquitoes in San Angelo and report on the diseases they may carry. To do this, mosquitoes were collected from two sites located along the Red Arroyo in San Angelo. One site was near Unidad Park and the other was along the Red Arroyo Trail. The mosquitoes were identified and the particular diseases that can be passed to humans and domesticated animals by these different species were researched. This information demonstrates the potential health issues that could occur in this area if the disease migrates to San Angelo. The project placed a greater focus on the public health aspect of mosquitoes as this perspective would shed light on the potential for future outbreaks and make people more aware of the risks and the important preventative measures they should use to protect themselves and their families.

MATERIALS AND METHODS

Mosquitoes were collected from two areas near the Red Arroyo in San Angelo, Texas (Figure 1). One site was at College Hills Unidad Park (Figure 2), and the other site was about a mile east on the Red Arroyo Trail (Figure 3). At each location, a mosquito trap (BG-Sentinel 2, Biogents AG, Germany) with CO₂ and a lure was used to capture these organisms. Both CO₂ and a lure were used to maximize the number of species captured by the trap since some mosquitoes are only attracted to one or the other.⁵⁵ The lure consisted of a commercial lure (BG-Lure, Biogents AG, Germany) or a freshly worn sock. The sock and commercial lure act as attractants for the mosquitoes. Specifically, scent from the sock and the chemical mixture in the lure serves to selectively entice these organisms to the source of the human odor. Once attracted to the area of the mosquito trap opening, the fan within the trap brings the mosquitoes inside a collection bag. The trap was generally deployed at a location an hour before sunset, and the trap and mosquitoes were collected a couple hours after sunset. The mosquitoes were frozen in an average home freezer until dead and stored in glass vials on the counter of the parasitology lab at Angelo State University. Mosquitoes were collected from mid-October through the beginning of November 2016.

In the lab, mosquitoes were identified using the procedures outlined in Darsie and Wards's (1981) *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. This text is a mosquito identification book specific to North American species and only addresses the female sex.⁵⁶ The identification process included viewing these mosquitoes using an Olympus stereomicroscope, which possesses a camera for

taking pictures of the mosquitoes. This not only helped with the identification process by allowing viewing of key morphological features that distinguish the genera and species, but it also allowed photographs to be captured of different mosquitoes. Some of the features used during the identification process include the metapostnotum setae (present or not present), the venation pattern on the wings, the maxillary palpus length compared to the proboscis length, leg banding patterns, leg segment proportion, scaling pattern on the thorax, setae presence or absence in front of and/or behind the spiracle, setae pattern on the dorsal side of the thorax, alignment of thorax structures, wing coloration patterns, and many more characteristics.⁵⁶ Some of these key structures are shown in Figures 4-7.

After identifying all of the mosquitoes, research was done on the various species that were found. Literature searches were performed online and through printed sources in order to record the specific diseases the mosquito species could possibly carry and transmit to humans and/or domesticated animals. Then, this information was used to describe these diseases in more depth, including discussing the pathology that could result in humans and/or domestic animals.

Also, Tables 1 and 2 were created by using the Texas A&M University Agrilife Extension website by searching for mosquitoes found in Texas and clicking on all of the linked species in order to see if they appear in Tom Green County on the provided state map.¹ This was done in order to provide readers the mosquito species found in this area in this study and those species reported by other individuals. This also provided for a more

inclusive presentation of the possible diseases that could infect humans and domestic animals in this county.



Figure 1. Sites of collection as presented on Google Earth.⁵⁷



Figure 2. Unidad Park collection site as presented on Google Earth.⁵⁷

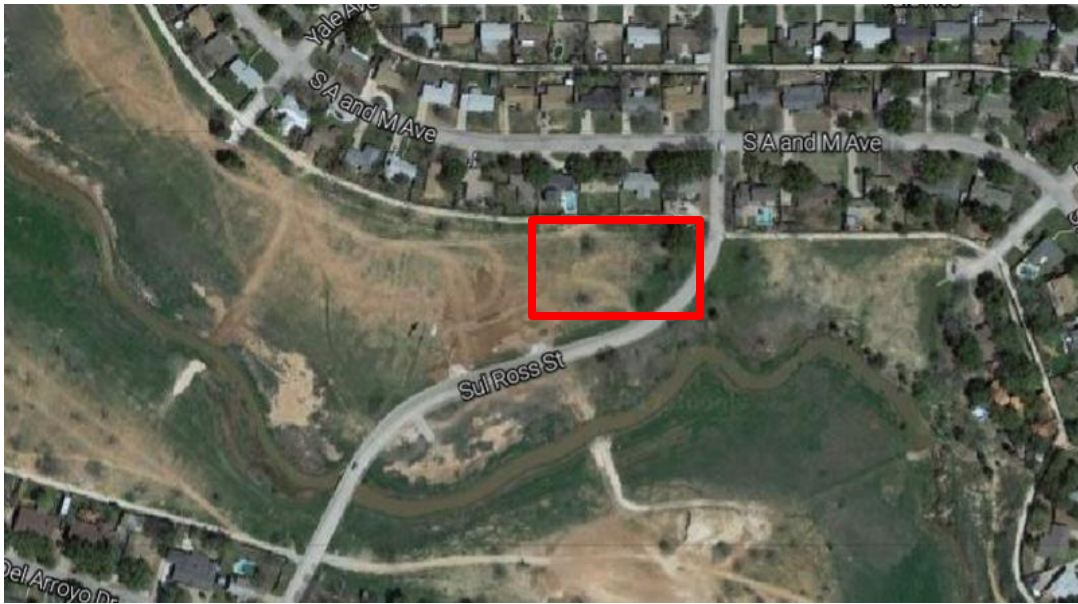


Figure 3. Red Arroyo Trail collection site as presented on Google Earth.⁵⁷

RESULTS

During part of October and November of 2016, 268 mosquitoes were collected in 13 trap-nights. Some collections captured fewer than 10 individuals per evening while others captured over 100 individuals per evening. Most of the mosquitoes collected were identifiable and resulted in identification of 11 species. Specifically, I found several species in the *Anopheles* genus (*An. pseudopunctipennis*, *An. judithae*, *An. punctipennis*, *An. crucians*, *An. perplexens*, *An. barbei*, and *An. atropos*), *Psorophora columbiae*, *Ochlerotatus hendersoni*, *Mansonia titillans*, *C. erraticus*, and many mosquitoes that could not be identified (Table 2). The most common species recovered include *C. erraticus* and *An. pseudopunctipennis*. Figures 4-7 show a few examples of the mosquito species collected and some of the key morphological features used to identify them.

Table 2. Mosquito species collected and the diseases they are reported to carry.

Species	Number collected	Diseases reported to be vectored
<i>Anopheles atropos</i> *	6	Malaria ⁴ ; West Nile virus ⁶
<i>Anopheles barbei</i> *	2	Malaria ⁴ ; West Nile virus ⁶
<i>Anopheles crucians</i> *	3	Malaria ² ; dog heartworm ⁵ ; West Nile virus ⁶
<i>Anopheles judithae</i> *	7	Malaria ⁴
<i>Anopheles perplexens</i> *	1	Malaria ⁴
<i>Anopheles pseudopunctipennis</i>	10	Malaria ⁴
<i>Anopheles punctipennis</i>	1	Dog heartworm ⁵ ; malaria ⁴ ; West Nile virus ⁶
<i>Culex erraticus</i>	121	Eastern equine encephalitis ² ; St. Louis encephalitis ² ; West Nile virus ⁶
<i>Mansonia titillans</i> *	1	Venezuelan encephalitis ² ; West Nile virus ⁶
<i>Ochlerotatus hendersoni</i> *	1	None reported
<i>Psorophora columbiae</i>	1	Dog heartworm ⁵ ; West Nile virus ⁶
Unidentifiable	114	(see page 30 for explanations)

*New location record for Tom Green County, TX



Figure 4. Identifying *Culex erraticus*. Note the lack of setae on the metapostnotum (red arrow).

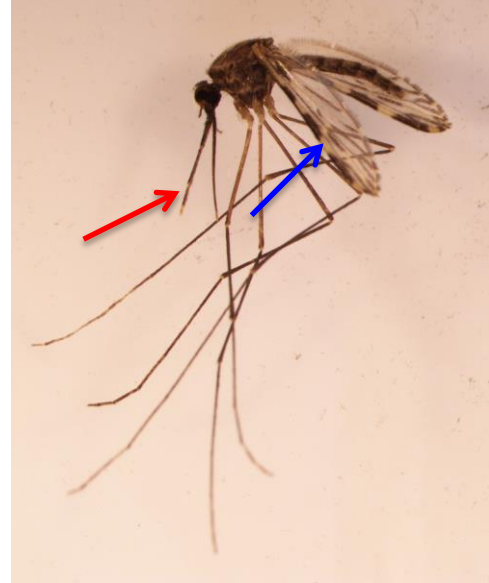


Figure 5. Identifying *Anopheles pseudopunctipennis*. Note the pattern on the wings (blue arrow) and the equal length of the maxillary palpus and the proboscis (red arrow).



Figure 6. Identifying *Anopheles crucians*. Note the equal length of the maxillary palpus and proboscis (red arrow).



Figure 7. Scaling pattern of the thorax of *Culex erraticus*. The pattern (red circle) is an important trait for this species.

DISCUSSION

The San Angelo area is home to a diverse group of mosquitoes. During late Fall 2016, 11 different species were recovered from two locations near the Red Arroyo. Four of the 11 species have been previously reported to occur in Tom Green County,¹ which means seven species are newly recorded in the county (Table 2). The most common species found was *C. erraticus* representing 79% of the individuals identified. A large number of individuals were unable to be identified. This lack of identification occurred for varying reasons, such as the inability to view the proper mosquito morphology that would have allowed identification. Specifically, some mosquitoes had missing limbs, others were covered with obstructive fungi, and the remaining looked as if decomposition of the bodies had occurred. In retrospect, it would have been advantageous to use a desiccant after freezing and killing the mosquitoes because at the time of collection there had been a period of rainy days. Collecting soon thereafter, when the air was still humid, may have generated the perfect fungal growth conditions during the storage process. Also, some difficulty in the identification process may have arisen because sex was not accounted for in the collections—only species identification was of concern. It is possible to have caught some male individuals because of their attraction to the females. This is important because only female mosquitoes (and larvae) distinguish the various species.⁵⁶ Thus, examination of a male would have led to the individual being classified as “unidentifiable.”

The identified mosquitoes *P. columbiae*, *An. punctipennis*, and *An. crucians* were found to be carriers of *D. immitis* in the states of Florida, Louisiana, and Georgia.⁵

Dirofilaria immitis is a filarial nematode parasite that lives in the heart and pulmonary artery of canines, hence its common name of dog heartworm.^{53,54} Pathology in the dog includes organ failure, heart failure, and lung disease. To combat this debilitating and potentially fatal parasite, it is recommended that pet dogs take monthly antihelminthics to prevent establishment of the adult worm.⁵³ Monthly administration may not be required in northern states where mosquitoes are dormant during cold months, but year-round transmission may occur in areas like San Angelo considering the mild winters and the possibility that mosquitoes are feeding during the warmest days of the winter. *Culex erraticus* is another species that could possibly act as a vector for this parasite based on a study performed in Oklahoma. According to that study, the evidence is weak but sufficient to warrant further investigation.⁵

Based on past researchers' work and this study's collections, it can be deduced that the West Nile virus can be transmitted by seven of the 11 species identified. This is a substantial number, especially considering that only 20 species of mosquitoes were reported in the area, eight of which have been reported to act as vectors for this virus (Table 1). Moreover, this project adds seven mosquito species to the list of those known to occur in Tom Green County, TX, and four of these are reported vectors of West Nile virus. The total number of vectors is now 12 species. From this information it is fair to infer that there is a risk of becoming infected with the West Nile virus for residents or visitors to this area. There may be several reasons why this disease is not a large concern in the local community, including but not limited to, the subtle nature of the symptoms this disease elicits and the

possibility that there is a reservoir host holding this virus instead of infecting the human population. Regardless of the reason for limited concern, it is still invaluable to be aware of the presence of West Nile virus in San Angelo, Texas. Without being aware of this mosquito-borne disease, an individual may mistake the potentially fatal West Nile virus as simply the cold. The idea of a reservoir host is also of great importance considering that, although the human population may not be experiencing this virus's ill effects, there is still potential for its transfer to the community in the future. Additionally, *C. erraticus*, the mosquito species accounting for over half of the mosquitoes identified, was reported to act as a vector for this disease. Thus, West Nile virus is likely present in this area and the risk of being infected is possible even into November.

Two identified mosquito species, *C. erraticus* and *Mansonia titillans*, are reported to carry one if not multiple variations of the encephalitis viruses. For example, *C. erraticus* is reported to carry Eastern equine encephalitis and St. Louis encephalitis.² *Mansonia titillans* is reported to act as a vector for Venezuelan encephalitis.² The Western equine encephalitis, Eastern equine encephalitis, Venezuelan encephalitis, and St. Louis encephalitis vary in geographic distribution in the United States, age group most at risk of infection, mortality, sequelae, and symptoms presented. Western equine encephalitis is located in the western and midwestern region of the country, affects infants as well as adults older than 50 years, has a 5-15% mortality rate, moderate to low sequelae depending on age, and presents with headaches, seizures, and altered consciousness. The Eastern equine encephalitis is located along the eastern portion of the country along the Gulf coast and into the South. This

encephalitis affects children, has a 50-75% mortality rate, 80% of survivors exhibit sequelae, and presents with the same symptoms as the Western equine encephalitis. The Venezuelan encephalitis occurs in the southern portion of the United States, affects adults, and results in a 1% mortality rate. This virus rarely exhibits sequelae and presents with myalgia, headache, and pharyngitis.⁴⁰ This virus may appear to be asymptomatic or produce acute encephalitis—or anything in between. Children infected by the Venezuelan equine encephalitis experience more adverse effects than infected adults. For example, children tend to experience fatality as a result of the encephalitis or experience long-term neurological sequelae.⁷ The St. Louis encephalitis is located in the central, western, and southern regions of the country. This virus affects adults over the age of 50, has a 2-20% mortality rate, and 20% of survivors exhibit sequelae. The symptoms presented by those infected with St. Louis encephalitis virus include nausea, headaches, vomiting, irritability, stupor, and disorientation.⁴⁰

As previously mentioned equines, as noted in the name of the above viruses, act as hosts for these pathogens. These organisms are greatly affected by these infections and the various strains provide different presentations of symptoms. For example, enzootic Venezuelan equine encephalitis is avirulent to horses while epizootic strains result in high morbidity and mortality of these animals; the morbidity rate is about 40-60% of susceptible horses, and the mortality rate is about 50% in this virus's case. The symptoms these infected horses exhibit include depression, convulsions, leukopenia, weakness, and fever. Pathologically these animals develop meningoencephalitis, among other detrimental neurologic symptoms.³¹ Substantial concern exists for the domesticated horses and those

humans interacting with them that vaccines are given against, for example, the Venezuelan equine encephalitis. Also in times of increased infection disallowing passage of these horses into a country such as the United States can act as a safety measure against this particular virus.⁷

Malaria is a disease not normally associated with the United States, but because of the prevalence of travel and immigration from countries still battling this disease, there is still potential for infection. For example, this study discovered seven *Anopheles* species, and it is documented that this genus is able to transmit malaria.⁴ Finding this genus is valuable information for the citizens of San Angelo. It is important to be aware that even though this disease is not a common concern in the United States, it could potentially be in this country and even in San Angelo. All that is necessary to have clinical cases of this disease is to have an individual infected with malaria, such as an immigrant or traveler from a malaria-endemic area, come into this region and the *Anopheles* mosquito take a blood meal from this individual. This simple process could then lead to domestic cases of malaria and all of the debilitating symptoms that accompany this disease.

One species collected, *Ochlerotatus hendersoni*, has not been reported as a disease vector. Two explanations exist for this finding. These mosquitoes may have the potential to serve as a vector, but researchers have not reported them as such because experimental infections were not performed or screening for diseases did not occur during mosquito surveillance. Alternatively, this species might not serve as a vector for some unknown

reason. Several species of *Ochlerotatus* serve as a vector for West Nile virus, so the former may be the more likely explanation.³

The 11 mosquito species cumulatively found in this study are reported to carry six different viral or parasitic diseases (Table 2). These six diseases show fairly close consistency in diseases reported to be carried by mosquitoes in this region of the state, which is seven diseases (Table 1). The state of Texas is home to 85 reported mosquito species leading to a variation in species that could transmit these diseases and possibly more. As previously mentioned, the regional movement of mosquitoes and possible differences in species presence during different seasons both lead to further complications in the disease processes of these organisms, i.e. extended season for transmission and variability in the risk of infection. The limited time-span for this particular study's collections, combined with the number of mosquito species reported in Texas and the before mentioned migration ability of mosquitoes, means that the vectors necessary to transmit other diseases such as Zika may be reported in San Angelo over time.

A multitude of future directions could be supported by the information presented here. One such study includes a distributional analysis of mosquito species across the city of San Angelo. In essence, are all mosquito species distributed equally across the city, or are some species more common in certain areas? Also, a seasonal surveillance of mosquitoes, noting differences in the distribution of species collected, can reveal the location and time of year when citizens must be most vigilant and take protective measures to prevent infection by these mosquito-borne diseases. The big picture of this project would be to generate a health

risk map for the community of San Angelo. The risk map would indicate the areas of the city and time of year when citizens are most at-risk to acquire a vector-borne disease. Finally, continued, annual surveillance is recommended to document the possible movement of species and their pathogens into the area. This study adds seven new species to the mosquito community, but this survey only occurred during a few weeks of the year. Consequently, many more species may have been missed.

This research identified and is presenting some of the medical issues that could potentially develop in the population of San Angelo as a result of the mosquitoes found in the city. Hopefully, this information would influence individuals to take safety measures against mosquitoes to reduce the chance of acquiring vector-borne diseases. Also, this project may educate people to be more aware of these potentially harmful organisms in their population and realize that these common “pests” are actually more dangerous than they appear. With this in mind, it is important to be aware of preventative measures to keep safe from irritating mosquito bites and their possible consequences. For example, keeping the body covered with long sleeves, long pants, closed toe shoes, and hats is always a good recommendation. This coverage can protect the individual by preventing the mosquitoes from reaching skin to take a blood meal, and therefore, eliminating the possibility of becoming infected with a disease if the mosquitoes are hosting a pathogen at that time. Another prevention method is to use mosquito repellent sprays or lotions. These repellents are a good product to use to protect areas that are more difficult to cover with clothes, such as the hands and face. In some situations, such as recreational activities like playing soccer or swimming, full clothing

coverage is not possible and the repellent can serve as a way to still protect one from mosquito bites. Additionally, giving heartworm medications regularly and throughout the year is a good means to protect domestic dogs from developing heartworm disease since it is difficult to apply the preventative measures recommended for humans to our domestic pets. Finally, horse owners are encouraged to maintain vaccination of their horses against the equine encephalitis viruses.

The information presented can serve to educate the population on their daily health and livelihood. Large scale elimination of mosquito habitats, specifically wetlands, is a good option for some areas of the United States. But for areas where this is not possible or desired, the public should at least practice personal protection via repellent sprays and clothing coverage. Moreover, individuals should avoid the outdoors during peak mosquito season and avoid known mosquito habitats.² Individuals may also participate in programs similar to those promoted by the city of San Angelo, such as eliminating the breeding sites for these organisms. This activity includes unclogging gutters, turning over objects containing water, emptying and refilling items holding water like birdbaths regularly, and similar measures. Also, the city promotes other actions such as clothing cover, repellent use, checking for tears in screen doors or windows, and wearing pre-treated clothing.⁵⁸ Some of these methods may be cumbersome; however, the outcomes are worth the time and effort. A life without these many mentioned mosquito-borne diseases is a luxury in some countries, and with the United States' advanced monitoring, healthcare, and preventative methods, this should be considered a doable task.

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BIOGRAPHY

Mattie Price was born in Lancaster, Texas, and was raised in the small central Texas town of Salado. She graduated within the top 10% of her class from Salado High School, deciding to continue her education at Angelo State University with a focus in biology. She was accepted into the Honors Program at ASU where she was introduced to a multitude of advantageous opportunities. The ideas and courses she was exposed to through this program and the biology department allowed her to confirm her ambitions of becoming a physician.

After graduating with her bachelor of science in biology from ASU, Mattie will return to the central Texas area to perform research at the Texas A&M Health Science Center. She will be doing this while she pursues her end goal of becoming a physician.

Questions or comments may be addressed to the author at: mprice12@angelo.edu