Fall 1-6-2017

An Epidemiological Study of West Nile Virus in Maricopa County, Arizona

Shawna Bronte

ABSTRACT

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Introduction: Vector-borne infectious diseases represent a major public health problem in both developing and developed nations. In particular, West Nile Virus (WNV), a mosquito-borne disease that can lead to severe disease and death in humans, caused over 2,100 reported cases in the United States last year (CDC, 2016). In Maricopa County, Arizona WNV has caused 474 reported cases during the last five years, with a case-fatality rate at 7.8%.

Aim: To examine the association between weather patterns and incidence of WNV in Maricopa County, AZ from 2007 to 2013.

Methods: We analyzed weekly data on climatological variables and WNV incidence from Maricopa County, AZ. The specific independent variables of interest were precipitation, minimum temperatures, mean temperatures, and maximum temperatures. A full model was generated using multiple linear regression, and a stepwise selection procedure yielded a minimal model.

Results: The full multiple linear regression model explains 45.30% of the observed variance in WNV incidence. The variable showing a significant impact on WNV incidence in this model was rainfall ($p < 0.0001$). Stepwise selection results explained 45.16% of the variance observed in the data. This model included two significant predictors: precipitation and maximum temperature.

Conclusion: Climatic variables, particularly the amount of rainfall and maximum temperatures, significantly influence WNV dynamics in Maricopa County, Arizona. These findings are in line with prior studies and could be useful to guide mosquito control programs in the state of Arizona.

AN EPIDEMIOLOGICAL STUDY OF WEST NILE VIRUS IN MARICOPA COUNTY, ARIZONA

by

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B.Sc., GEORGIA SOUTHERN UNIVERSITY

A Thesis Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the
Requirements for the Degree

MASTER OF PUBLIC HEALTH

ATLANTA, GEORGIA
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APPROVAL PAGE

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Date: November 28, 2016

ACKNOWLEDGEMENTS

I would like to thank my mentors for all of their guidance and patience during this process. They helped me arrive at this point in my life. Additionally, I want to acknowledge my wonderful husband for all of his support and encouragement throughout these years of stress. Without my husband to advise and encourage me, I would have never pursued this field of study. I am very lucky to have someone like him in my life, and I know I cannot thank him enough for all that he does for me.

AUTHOR'S STATEMENT

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Shawna Bronte
Signature of the Author

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CHAPTER I: INTRODUCTION

1.1 Background

West Nile is a vector-borne disease in the group of arboviruses, which contains those illnesses that are transmitted by an arthropod vector. West Nile Virus (WNV) was identified for the first time in the West Nile District of Uganda in 1937 and belongs to the *Flaviviridae* family, as does Dengue fever and yellow fever. However, West Nile virus is typically transmitted by mosquitoes belonging to the *Culex* genus, whereas Dengue and yellow fever are transmitted by mosquitoes of the *Aedes* genus (Roehrig, 2013). These viral infections are distinguished from one another through targeting specific genetic proteins in laboratory and/or clinical settings (Wong et al., 2003). This particular virus is transmitted between avian hosts and mosquitoes, but has the potential to be transmitted to horses, humans, and other mammals. Birds serve as natural reservoirs for the WNV transmission cycle. Humans, horses, and other mammals are dead-end, or incidental, hosts. A dead-end host is one that cannot amplify the virus enough for further transmission to occur (DeGroote, Sugumaran & Ecker, 2014). This virus is endemic to numerous countries in Africa, West Asia, and the Middle East, but has recently established itself in the Americas. Nations such as the United States, Canada, Mexico, countries in Central America, and the West Indies have become familiar with WNV. The virus became recognized as a cause of severe human infection during an outbreak in Israel the 1950s. After that situation had ended, the virus was quiet for several decades before the Romanian epidemic of 1995 (Roehrig, 2013). To date, WNV has been reported across the globe (Soverow, Wellenius, Fisman & Mittleman, 2009).

As a virus that makes use of the Host-Agent-Vector-Environment relationship, its spread did not come as a great surprise. The first cases of West Nile Virus in North America occurred in 1999 in New York City. Origins of the virus and its introduction into the United States remain unknown. When WNV was first recognized in Europe, it was traced back to the strain circulating in Africa at the time. This spread was attributed to bird migratory patterns (Mann et al., 2013). However, when WNV appeared in the United States, it was inexplicable. The pure fact that the virus moved from the Eastern hemisphere into the Western hemisphere and manifested across two continents in under ten years shows that it is a definite public health threat. The initial emergence of WNV in New York has been recognized as a major event in arbovirology during the last two hundred years (Roehrig, 2013). After the early cases in New York, other states in the U.S. began to report WNV incidence. According to the CDC, in 2000 New Jersey and Connecticut experienced the first cases in their state. By 2001, additional cases of WNV were reported in other northeastern states and some southeastern states. The year of 2002 brought the largest spread of WNV seen in United States history, with new case reports from over 20 states. By 2003, there were only five states that had not seen the virus among the population. Oregon saw the first human infections in 2004, and cases began in the state of Washington in 2006. Maine, Alaska, and Hawaii were the only states that had not experienced any WNV cases in humans by 2008 (CDC, 2010).

Figure 1 reveals the dissemination of WNV in the United States from the initial cases through 2006. As indicated by the color of Arizona in Figure 1, WNV began to spread in the state in 2003. During the first year, Arizona only experienced 13 cases. However, between 2004 and 2006, the case count reached 654 with a case fatality rate of approximately 4.9 percent. Across the United States as a whole, the case fatality rate between the same years was about 4.0 percent (CDC, 2015). This difference was also observed during the study interval. In Arizona, the case fatality rate between 2007 and 2013 was nearly 7.1 percent while the national case fatality rate was only 4.5 percent (CDC, 2015). In addition to these statistics, literature studies surrounding WNV and the factors influencing its spread will be considered in the next chapter of this work.

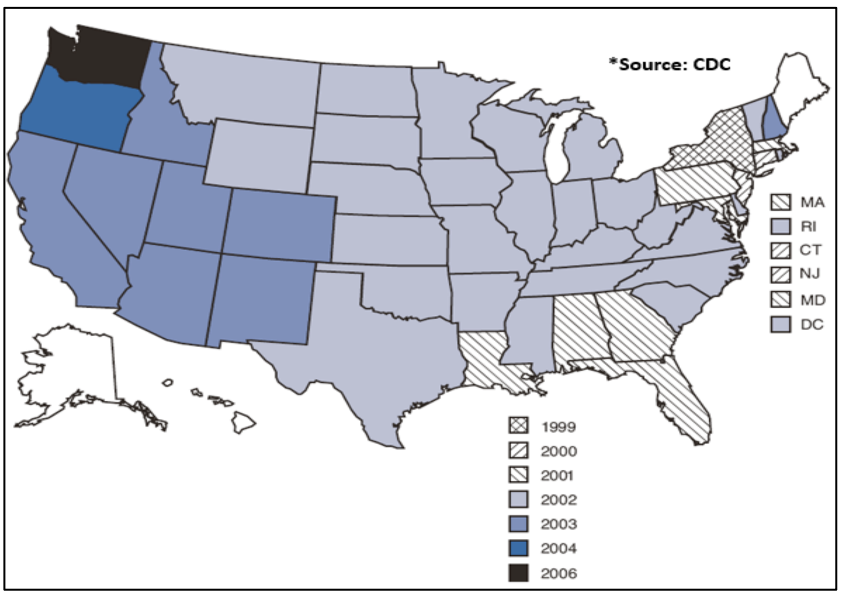

Figure 1. The spread of WNV among humans within the U.S., 1999-2006
Map of the United States showing when human infection with West Nile virus was first reported from each state between 1999 and 2006 (CDC, 2010). New York was the first state to experience human infections. To date, West Nile virus has not infected any humans in Alaska or Hawaii (CDC, 2016).

1.2 Purpose of the Study

Factors potentially influencing the distribution of WNV incidence have been identified by multiple authors in the literature. Such studies indicate that local climate factors have an impact on the viral spread, but not all authors agree on which aspects of the climate are leading to differences in incidence (Epstein, 2001; Montgomery & Murray, 2015; Deichmeister & Telang, 2011; Morin & Comrie, 2013). Any temporal patterns that emerge from the present analysis of WNV in Maricopa County, Arizona will be modeled to account for the climatic variables such as temperature variance and rainfall influences on viral transmission. The intent of this thesis is to determine how the climate of Maricopa County, Arizona impacts the WNV incidence seen in the area for years 2007 to 2013 at the population level. It is hypothesized that higher than average temperatures and above average precipitation will allow the vector to breed more frequently, increasing the vector population size and leading to an increased incidence of West Nile Virus. This hypothesis is based on findings from other authors (Hanh et al., 2015; Wimberly, Lamsal, Giacomo & Chuang, 2014). The hypothesis will be tested by identifying the effects, such as temperatures and precipitation, establish to enhance the vector(s) ability to influence the incidence of West Nile Virus. In addition, examination of any confounders of the association between WNV incidence and climatic patterns has to take place. While both the climate data and data about WNV cases are openly available, little information regarding a connection between these two factors has been presented in the literature to our knowledge thus far. The literature review will discuss previous research on WNV incidence and climate factors that may influence this incidence. Chapter III will explain the methodology and the procedures used to conduct the analysis of WNV incidence and climate-related factors based on aggregated climate data and incidence reports. In Chapter IV, the outcome of the study will be presented. Any appropriate tables or figures will be used to display temperature differences, rainfall measures, and case counts. The last chapter will discuss additional details discovered in this study, expand upon the research questions and offer suggestions about climate and WNV incidence based on the findings.

1.3 Research Questions

Determination of the association between the climate factors and West Nile Virus will be achieved by answering the following questions:

1) What climatic patterns are observed in Maricopa County between 2007 and 2013?
2) How can the contribution of varying climatic conditions be quantified in terms of their impact on the incidence in this county?
    a. Standard statistical regression analyses

CHAPTER II: REVIEW OF THE LITERATURE

This thesis will explore the changes in the incidence of West Nile Virus in Maricopa County, Arizona during the aforementioned years. The literature review presented here describes the current knowledge in the field surrounding climate factors and WNV incidence. Specifically, a brief synopsis of the viral transmission cycle, the clinical characteristics of WNV, genealogy, prevention methods, and previous work related to the climate and WNV is made available.

2.1 WNV Transmission Cycle

There are a few relevant terms to learn if one wishes to understand the Host-Agent-Vector-Environment relationship of WNV. For example, the extrinsic incubation period is the time from ingestion of the infectious blood meal until a female mosquito can transmit the infection. Another important term is the gonotrophic cycle. This cycle measures the time between a female taking a blood meal and the female laying her eggs before searching for another blood meal. Finally, in terms of WNV, vector competence refers to the capability of the mosquito to acquire, maintain, and transmit the virus. For maintenance of the virus, the *Culex tarsalis* mosquito must live in temperate or tropical regions of the world. This is explained by the viral ambient temperature required for replication ranges from 50 to 60 degrees Fahrenheit (Reisen, Fang & Martinez, 2006).

This infectious disease has illustrated its ability to infect multiple competent vectors and other animals, including horses, squirrels, and humans, but the scientific community agrees that birds are the primary reservoirs of West Nile (Liu et al., 2009; Epp, Waldner, West & Townsend, 2007). Despite the high number of avian species that have tested positive for WNV, only those species that develop adequately high levels of viremia will be able to transmit WNV to mosquitoes. These hosts are called the amplifying hosts. For any mosquito to acquire West Nile Virus, it has to bite an infectious animal. This enzootic transmission cycle between birds and mosquitoes is essential for the virus to spread. Given that not all birds can successfully transmit WNV to mosquitoes, an increased avian diversity in a geographical area has the ability to render a decreased WNV incidence for that area (Allan et al., 2008; Mann et al., 2013).

In the U.S., crows are of particular importance due to their high susceptibility to West Nile Virus. Crows develop a severe illness, and their mortality rate is high, which makes them useful as sentinels for the presence of a virus in a new endemic area. For example, crows were the deciding factor when West Nile Virus was first investigated in New York in 1999. The preliminary events of the outbreak were determined to be part of another St. Louis Encephalitis virus (SLEV) resurgence. However, when the number of crows dying continued to increase over the following days, the outbreak was clearly differentiated from the suspected SLEV (Roehrig, 2013). In the state of Arizona, a study was conducted during the summer of 2010 to discover the essential viral amplifiers in the Phoenix area. Maricopa County encompasses Phoenix and the surrounding cities of Arizona. The study revealed that the majority of local birds infected with WNV were either house sparrows, (*Passer domesticus*), house finches (*Haemorhous mexicanus*), or great-tailed grackles (*Quiscalus mexicanus*). Understanding the importance of these species in terms of WNV transmission is essential for the residents of Maricopa County. Specifically, these species are common in the area and may be observed in a resident's backyard during any season of the year (Komar, Panella, Young, Brault, & Levy, 2013).

Figure 2 illustrates the transmission cycle of WNV used by both the CDC and the Maricopa County Department of Public Health. When a female mosquito first feeds on an infected avian host, the virus incubates in the mosquito's body for around ten days (Daep, Munoz-Jordan, & Eugenin, 2014). After the extrinsic incubation period passes, the mosquito will be able to transmit WNV to new birds and other hosts, including humans, for the rest of its life. The variance in the number of times a female mosquito takes blood meals during her life depends on the length of the gonotrophic cycle. For humans to contract the virus, they must interact with the vector and be bitten. Thus, it should not be surprising that human population density has been shown to strongly correlate with the prevalence of the virus (Allan et al., 2008). Once the virus enters the human body, the infected individual begins to see the symptoms of WNV infection within one week and the entire period of infection ranges from 3 to 14 days for most cases. Even asymptomatic cases can serve as sources of transmission at times (Daep, Munoz-Jordan, & Eugenin, 2014). This is possible since an uninfected mosquito may still feed on an infected, asymptomatic human. In this manner, humans can indirectly contribute to WNV transmission and spread.

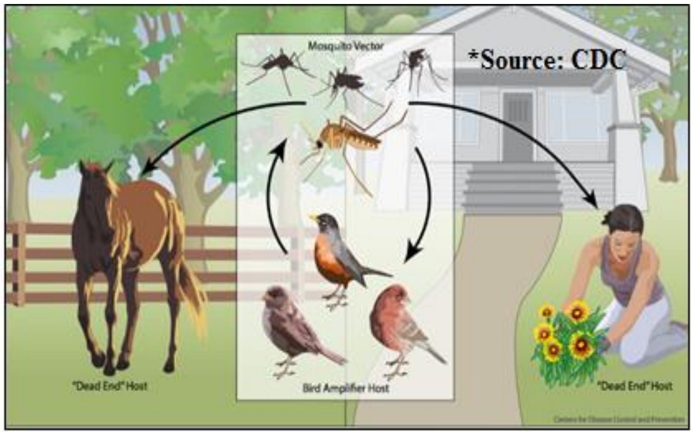

Figure 2. Transmission Cycle of the West Nile Virus
Illustration of the basic transmission cycle of West Nile virus. The virus naturally circulates between mosquitoes and avian hosts. Mosquitoes transmit the virus to other hosts, including humans and horses, by taking a blood meal from the host. Infectious humans can indirectly serve as sources of infection for uninfected mosquitoes.

2.2 Clinical Characteristics of WNV

Vectors under study for this research are those belonging to the *Culex* genus because they are the most common mosquitoes in the area known to transmit WNV to humans. Symptoms of West Nile Virus infection can vary in severity. The majority of people who become infected with WNV will never exhibit symptoms or even be aware of the infection. Due to this, the number of cases of WNV in a given location is thought to be drastically underestimated (Gardner, 2009). West Nile Virus is known for leading to symptoms in about 20 percent of those infected and can also result in neuroinvasive disease (Gardner, 2009). While less than one percent of individuals with WNV develop neuroinvasive disease, it impacts the nervous system of the individuals and includes West Nile meningitis, West Nile encephalitis and West Nile myelitis (Hahn et al., 2015). West Nile Virus is usually transmitted to humans through the bite of an infected mosquito, or in very rare occasions, through blood transfusion, breastfeeding, and transplantation. For those who are infected and become symptomatic, it is typical to experience fatigue, headaches, fever, body aches and swollen lymph nodes. Symptoms occur two to fifteen days after a mosquito bite and usually last a few days but can last up to several weeks. Individuals who develop neuroinvasive disease typically have symptoms such as a high fever, stiff neck, muscle weakness, headache, tremors, disorientation, coma, convulsions and paralysis (Montgomery & Murray, 2015). These patients take longer to recover from the illness than do those with West Nile Fever. Patients with West Nile fever tend to normalize in about one year, whereas those with the neuroinvasive disease suffer for longer periods of time (Kulkarni et al., 2015).

2.3 Taxonomy of WNV

While there are at least seven separate strains of West Nile Virus circulating worldwide according to ample literature sources, those that infect humans are the only lineages of interest for this thesis. Thus, isolates of West Nile Virus fall into two major genetic lineages, lineages 1 and 2 Shah-Hosseini, Chinikar, Ataei, Fooks & Groschup, 2014; Magurano et al., 2012). Figure 3 shows these lineages and was produced by Monaco et al. in 2009. According to their phylogenetic analyses based on specific serological and virological information about the E protein of the virus, lineage 1 can be divided into four sub-clusters: 1A, 1B, 1C and 1D (Monaco et al., 2009). Sub-cluster 1A included strains traced back to outbreaks in Senegal in 1993, Romania in 1996, Italy in 1998 and 2008, Kenya in 1998, Bulgaria in 1999, and Morocco in 2003. The Israeli strain traced to 1998 and American strains from 1999 and 2000 were categorized into sub-cluster 1B. The third sub-cluster, 1C, has strains from Algeria in 1968, Egypt in 1951 and France in 1965. Finally, sub-cluster 1D contained the Kunjin virus traced to 1960. Lineage 2 is endemic in Madagascar and Southern Africa, but known to circulate in parts of Europe as well. In the study, the strains in lineage 2 originated in several nations. For instance, the strains were traced back in time to Uganda in 1937, Madagascar in 1988, Senegal in 1990, and Central Africa in 1983 (Monaco et al., 2009). Despite the fact that these strains infect humans, as incidental hosts humans, horses, and other mammals do not contribute at all to the genetic evolution of WNV (Duggal et al., 2015).

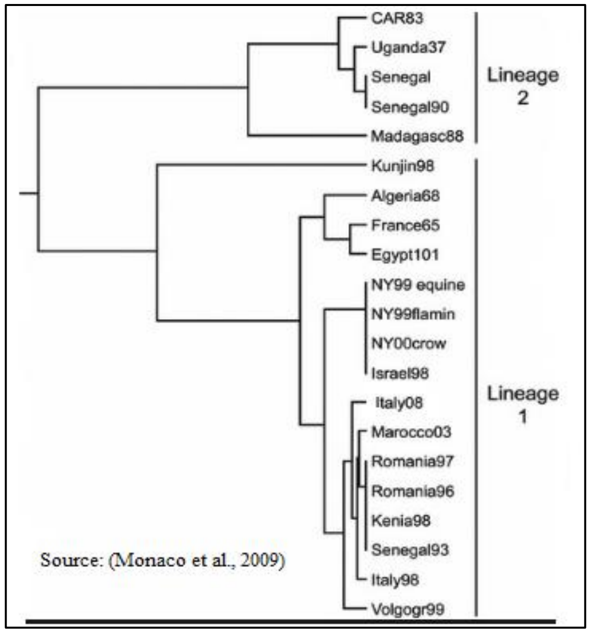

Figure 3. WNV Lineages known to infect humans
Human cases of West Nile virus are known to result from infection with lineage 1 or lineage 2 of the virus. The phylogenetic analysis shows that lineage 1 can be divided into four sub-clusters (1A, 1B, 1C, 1D) based on outbreaks over time. Lineage 2 is separated from lineage 1 by observed differences in the E protein of the virus and sources of historical outbreaks.

The virus itself is composed of a protein coat, or capsid, which houses the single-stranded, positive-sense RNA genome. Outside of the capsid is the outer envelope that is made up of proteins, lipids, carbohydrates, and trace metals (Colpitts, Conway, Montgomery & Fikrig, 2012). Multiple protein structures are explained in the literature, but such proteins and detailed genetics are beyond the scope of this thesis. However, the mosquito is also known to show some immune response to the virus via one of two known pathways: the innate immune pathway and the RNA interference (RNAi) pathway. The former route has been illustrated to have a role in controlling infection rates in mosquitoes, suggesting some antiviral immunity. The RNAi pathway has been connected to suppression of the mosquito immune response. An analysis of the WNV vector *Culex quinquefasciatus* revealed that some of the mosquito's genes are upregulated once the mosquito is infected. For instance, there is evidence of upregulation of the metabolism and transport of cellular materials (Colpitts, Conway, Montgomery & Fikrig, 2012). Therefore, it is important to keep the life cycle of mosquitoes in mind while also recalling the importance of the Host-Agent-Vector-

Environment relationship.

One study considering this relationship looked at the differences in terms of entomological risk of infection as the vector develops. The authors developed the Dynamic Mosquito Simulation Model. This model consisted of parameters that included aquatic development, egg laying and development rates, gonotrophic progression rates, vector competence, extrinsic incubation periods, mortality rates, and local weather data. This model is parameterized to particular species, and mosquito development rates were estimated for each life stage based on daily mean temperatures. The authors concluded that the number of mosquitoes present in the mid-summer has declined due to increases in temperatures (Brown, Childs, Diuk-Wasser & Fish, 2008). The model may serve as a framework for others who wish to study WNV or WNV surveillance strategies as well.

2.4 Importance of Surveillance

In the United States, the Centers for Disease Control and Prevention (CDC) has the responsibility of keeping the disease surveillance programs functioning. The CDC has programs in place to track any threats to health in the U.S., the leading causes of death, ease of healthcare access and differences in health outcomes across regions and populations of the United States. Infectious disease surveillance, such as WNV surveillance, tracks courses of potential outbreaks based on the analysis and interpretation of large datasets from a wide range of sources. This involves both epidemiological surveillance and environmental surveillance (CDC, 2016).

First, one must consider human surveillance, which is also a part of epidemiological surveillance. This type of surveillance is used nationwide and allows researchers to discover the routes of transmission WNV may use, how the virus presents in terms of clinical manifestation, and the ways to evaluate and diagnose the illness. Typically, the diagnosis can be confirmed through the detection of anti-WNV immunoglobulin M (IgM) antibodies in serum or cerebrospinal fluid of the patient (CDC, 2016). Passive human surveillance relies on local health officials to report cases that arise over time. Due to underreporting and incomplete diagnoses at the local level, WNV incidence is underestimated. To attempt to counteract this underestimation, environmental surveillance encompasses the majority of WNV surveillance activities used in the United States.

Vector surveillance, or mosquito-based WNV surveillance, can be defined as the process of collecting mosquito samples and screening them for arboviruses. The primary objectives involved are collecting data on the mosquito abundance and virus infection rates in an area, providing indicators of the potential for an outbreak, identifying geographical locations with increased risk of outbreaks, and monitoring how effective the surveillance system is at controlling the vector population. The overall number of vectors in a given area is termed the vector abundance and this number may change over time and in different locations (Sinka et al., 2016). The *Culex tarsalis* vector thrives in the temperate climate of Arizona, closely followed in terms of abundance by the *Culex quinquefasciatus* vector. While data on vector abundance is not available for the complete duration of the current study, the information was publically available for four years. In Maricopa County, AZ, vector abundance was monitored in terms of vector species and the number of positive pools. A positive pool, or positive sample, is defined by the presence of mosquitoes that test positive for WNV in the sample. Over 750 traps are placed throughout the county and collected once a week. The available data on *Culex tarsalis* spans from 2007 through 2010. Vector abundance is provided in terms of the number of positive pools from the county mosquito traps. If there is a high number of positive pools, it is suspected that there is also a high number of mosquitoes in the area that are competent West Nile vectors. In Figure 4, the graph illustrates the number of pools with *Culex tarsalis* and *Culex quinquefasciatus* mosquitoes that tested positive for West Nile virus between 2007 and 2010. This method of measurement is used as a proxy for estimating the true number of individual mosquitoes in the area.

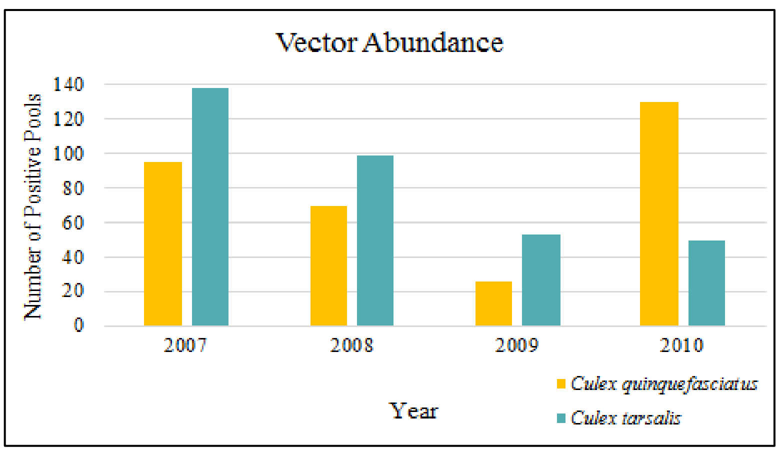

Figure 4. Vector Abundance in Maricopa County, 2007-2010

Graph of mosquito pools with vectors that tested positive for West Nile virus. Culex quinquefasciatus vector pools are shown separately from the Culex tarsalis vector pools since both species are known to transmit WNV in Maricopa County, AZ. Culex tarsalis is the most competent vector in this environment, as illustrated by the differences in the numbers of positive pools prior to the 2010 outbreak. Data was obtained from the Maricopa County Department of Public Health.

Mosquito surveillance conducted nationally by the CDC allows for results to be obtained relatively quickly (minutes to a few days), and offers a long-term baseline of historical data for risk assessments and computation of infection indices. However, this type of surveillance is not perfect. If infections rates are low, the virus may not be detected at all. Surveillance programs across the nation cannot be entirely universal due to differences in geography and climate, as well as differences in the number and types of mosquito traps that are used (CDC, 2016). An appropriate sampling of adult mosquitoes should be done weekly by trapping at fixed locations in the community that represent the types of habitats available in the region. Most of the commonly used traps for WNV surveillance sample host-seeking mosquitoes or gravid (carrying eggs) mosquitoes searching for an oviposition site or a place to lay her eggs. The infection rate in a vector population approximates the prevalence of WNV-infected mosquitoes in the population and is generally a good indication of human risk (CDC, 2016). Mosquito surveillance can provide insight on thresholds for proactive efforts toward vector control. It can also be used to show when proactive control measures were not sufficient at hampering viral amplification or when aerial spraying of mosquito adulticides was ineffective.

Along with human and mosquito surveillance, the CDC also outlines the guidelines for bird surveillance across the United States. West Nile virus is naturally amplified in nature through replication in bird populations. Aside from mosquito bites, birds can acquire WNV from eating infected prey (insects, small mammals, other birds) or from direct contact with other infected birds in rare situations. Even though several bird species may die from the infection, the ones that survive develop a life-long immune response. Detecting antibodies in young birds and dead birds is another potential pathway to monitor the transmission of WNV. All of these surveillance methods are combined in most cases to prevent human disease from occurring. Depending on the location and the amount of potential for human risk, a surveillance program is created to fit the situation. For instance, a single factor surveillance system may be the best choice. This kind of system only implements surveillance of one factor in the environment, such as a dead bird surveillance system or a sentinel surveillance system.

Some states in the U.S. have multiple factor surveillance systems and incorporate other environmental variables into their measures. In Arizona, state officials conduct both human and vector surveillance for West Nile virus. The surveillance data is reported at the county level. For each county, the number of confirmed and probable WNV cases are recorded and made available to the public on an annual basis. This data, published by the Arizona Department of Health Services, contains anonymized population demographics of the cases including race, ethnicity, gender, age and clinical presentation of the infection. Additionally, they publish the total number of mosquito pools in each county that is found to have WNV positive mosquitoes and the number of other animals that test positive for the virus. Such systems were developed after WNV reached New York for the first time. The national arboviral surveillance system (ArboNET) developed by the CDC and local health officials in 2000 was a direct result of the 1999 WNV outbreak. ArboNET has data on environmental factors, epidemiological findings and various arboviral infections seen in human viremic blood donors, non–human mammals, dead birds, sentinel animals, and mosquitoes. Surveillance systems like these play a significant role in preventing human infection with WNV (CDC, 2016).

2.5 Prevention Methods

Vector Control Measures

Mosquito control is either proactive or reactive. Proactive control has shown to work well to reduce WNV incidence in urban areas. Reactive control refers to implementing control measures after the occurrence of avian mortalities, which is usually not the best choice (Reisen & Brault, 2007). One of the biggest obstacles to overcome is overwintering of the West Nile Virus. Two researchers conducted a study to understand how the virus may withstand winter in North America. The authors of the study proposed four mechanisms by which WNV may be able to withstand extreme climates. One idea is that the female mosquito becomes infected before winter sets in, therefore the mosquito can transmit the virus once the temperature warms up in the Spring. This idea is feasible because WNV cannot replicate inside the mosquito unless the temperature is greater than or equal to approximately 14˚C (Reisen & Brault, 2007). Another mechanism these authors suggested is the migratory behaviors of birds. It is possible that birds leave parts of North America for the winter and bring the virus back to their native area when they return. The third proposal involves the idea of WNV being a chronic infection in birds. Since organs such as the kidneys of dead birds have tested positive for WNV in experiments, the plausibility of this concept is within reach. Finally, the researchers state that WNV transmission may simply continue through the winter in regions where the temperature rarely falls below 14˚C [or 57˚F]. Potentially, one or even all of these mechanisms may be contributing to WNV incidence in various regions of the world (Reisen & Brault, 2007).

Personal Protection

Simply speaking, the best way to prevent humans becoming exposed to WNV is to avoid all exposure to the mosquito or vector. In reality, avoiding mosquitoes is difficult if not impossible in most places. However, an individual can take precautions to reduce their personal risk of being bitten by mosquitoes. According to the recommendations from the Centers for Disease Control and Prevention (CDC), personal protective measures include wearing long sleeves and pants anytime the person is outdoors, using a DEET-based insect repellent on exposed skin and a Permethrin-based repellant on any clothing, as well as staying indoors during the peak hours of mosquito activity. This period usually takes place starting at dusk and continues until dawn the next day (CDC, 2006). Regardless of personal actions, vector control is vital for preventing the spread of WNV. A study published in 2015 highlighted the importance of mosquito surveillance systems in the United States. In 2004, such systems were federally funded, but in 2012, that funding was cut by 61 percent. As a result, there was an 18 percent decrease in the number of states using an active surveillance system. The decreased funding was a cause of concern because surveillance systems are often key aspects of detecting an outbreak before it becomes a large epidemic (Hadler et al., 2015).

Additional Prevention Measures

In addition to vector control and surveillance systems, any nearby storm drains or roadside ditches should be cleared of standing water. Any stagnant water can serve as an ideal and necessary breeding habitat for *Culex* mosquitoes in particular (Deichmeister & Telang 2011). Other sources of standing water that should be removed include swimming pools that are not in use and old tires. Another precaution to implement is early and consistent utilization of larvicides. If all else fails, the ultimate solution is the use of adulticiding, or using aerial dispersion to apply chemicals that kill adult mosquitoes (Epstein, 2001). If no vector control methods are in place, the number of infected humans will continue to rise. Mosquito control procedures are crucial to limiting disease spread, especially because there is currently no effective antiviral treatment or available vaccine for humans (Daep, Munoz-Jordan & Eugenin, 2014).

2.6 Related Works

<u>*Overview of the Literature*</u>

The present work is concerned with climatic factors and specifically concentrated on temperature and precipitation. This literature review includes numerous studies that span across time from 2008 to 2016. The years covered in the related work section were limited to this period to reflect the most recently published findings. Specific terminology was used to search through databases such as PubMed and EBSCO to ensure that all of the publications of interest were found. Keyword searches included the following list of terms: West Nile virus, mosquito, *Culex tarsalis, Culex quinquefasciatus,* mosquito control, temperature, precipitation, United States, climate change, environment, vector-borne illness, vector-borne disease, arbovirus, weather, environmental patterns, environmental variables, habitat, spatial or temporal distribution, Flavivirus, *Flaviviridae*, vaccine, Maricopa County, Arizona, horses, equine West Nile infection, avian hosts, West Nile infection in birds, human West Nile infection, risk factors for human infection, West Nile outbreak 2010, West Nile incidence, surveillance, public health, and West Nile epidemiology. Several researchers in the field have used personal risk factors and environmental factors to attempt to figure out regional WNV incidence patterns or create models to forecast the next outbreak (Brown, Childs, Diuk-Wasser & Fish, 2008; Chuang & Wimberly 2012). Risk factors for human infection with WNV include, but are not limited to old age, immunosuppression, and urbanization of the area of residence (Deichmeister, & Telang, 2011; Montgomery & Murray, 2015). Some of the most studied environmental factors include forest cover, wetland cover, vegetation, building footprints and climate measures (Brown, Young, Lega, Andreadis, Schurich & Comrie, 2015; Chen, Epp, Jenkins, Waldner, Curry & Soos, 2013; Chuang & Wimberly, 2012). The majority of the scientific community recognizes that global climate change is taking place and influences disease outbreaks (Soverow, Wellenius, Fisman & Mittleman, 2009).

As a matter of fact, by 2007 the Intergovernmental Panel on Climate Change had added vector-borne disease outbreaks to the ever-growing list of possible consequences of global warming (Paz, 2015). With a specific focus on the Host-Agent-Vector-Environment relationship, Paz published a review article discussing impacts of global climate change on the transmission of WNV. The author pointed out that outdoor temperature measurements have a positive correlation with the viral replication rates, growth rates of the vector population, efficiency of viral transmission from mosquitoes to birds and the geographical location of the human WNV cases. In addition, Paz determined precipitation measures, whether above or below the geographical average, were correlated with an increased potential for inducing a human outbreak of WNV. The researcher found that areas with above average precipitation records had higher numbers of mosquitoes, suggesting more viral transmission. Areas with below average precipitation, or areas of drought, often have a limited amount of standing water. WNV has been shown to spread quickly in areas of drought (Deichmeister, & Telang, 2011).The disease is able to spread because standing water attracts both mosquitoes and birds from the area, which forces an increased interaction between the vector and the natural host. As the amount of interaction increases, the epizootic cycle speeds up and amplifies the virus (Paz, 2015; Montgomery & Murray, 2015).

Related Works from different Nations

A comprehensive analysis, including five countries in the Mediterranean Basin and Central European region of the world, was used to determine the best ecological area for sustaining transmission of WNV. The analysis relied on data from Greece, Italy, Tunisia, Portugal, and Morocco during 2008-2012. Data included 270 clinical case records of human or equine infections. By using the Mahalanobis Distance Statistic and the Receiver Operating Characteristic (ROC) curve method, the authors of the study confirmed the validity of the sustainability maps that were produced. In temperate areas of the region, the mosquito vector had the highest rates of survival between May and November. However, in desert regions, the environment was suitable for mosquito survival between April and June (Conte et al., 2015). Even within a study of five countries and less than 300 cases, these researchers clearly distinguished geographical differences among the environments and transmission rates of WNV.

Another work, produced in Canada, examined three provinces (Alberta, Saskatchewan, and Manitoba) to forecast the spatial and temporal distribution of *Culex tarsalis* and future WNV infections. The model implemented the climate conditions from the provinces as of 2010 as the baseline input to predict the vector distribution and viral infection rate for the years of 2020, 2050 and 2080. All of the climate models used a median situation and two extreme conditions for each of the previously mentioned years. The study results across the models and years indicated that the northern distribution of the vector would double geographically, which aligns with the scientific evidence in support of global warming. In addition, the authors found that in locations where WNV is already endemic, the median infection rate in 2050 was 17.91 times the median infection rate seen in 2010 (Chen, Jenkins, Epp Waldner, Curry & Soos, 2013). If such forecasting and modeling of infections have been completed accurately, West Nile Virus may hold great potential for devastating outbreaks in the near future. These studies and the remainder of the studies discussed here are summarized and presented in Table 1 at the end of this section.

United States: National Level Studies

By performing a retrospective study, researchers from the United States discovered a significant spatiotemporal pattern as well. The pattern was found by examination of the relationship between WNV incidence in humans and county urbanization data from 1999-2006. Counties with less than 38 percent forest cover were of particular interest because they were also found to have a 4.4 times greater odds of WNV incidence exceeding 0.75 per 100,000 population. Within the eight northeastern states in the study, the urbanicity of the counties directly impacted the WNV incidence, with more urban counties seeing higher rates of infection (Brown, Young, Lega, Andreadis, Schurich & Comrie, 2015). A separate study of seven states in the northern Great Plains (NGP) region of the U.S. was based on satellite remote sensing data. Using this type of data from 2004-2010, the authors modeled WNV risk and environmental variation of land surface temperatures, normalized difference vegetation indices, and actual evapotranspiration. These variables were utilized because they are known to be sensitive to modifications in temperature and precipitation. Changes in these two climate factors were suspected to influence the dynamics of mosquito populations and WNV transmission. By controlling for the three aforementioned environmental conditions and modifying only temperatures and precipitation measures, the model provided precise predictions about the low WNV incidence observed in 2011 (Chuang & Wimberly 2012).

An interesting study of regions within the United States revealed differences between the northern and southern geographical areas. The authors of the work determined similar outcomes in the northern states but found that the southern region of the U.S. has become warmer over time and mosquito habitats have begun to dry out. The habitat changes will lead to earlier death of the mosquitoes and accordingly, a reduction in the number of human cases of infection. Researchers involved with the study also concluded that the southwestern areas will observe a delayed mosquito season due to the high temperatures in the Spring and Summer and the increased amount of rainfall in late Summer and Autumn (Morin & Comrie, 2013). In another research investigation, the authors found a weak association among temperatures in the Spring and Summer and human WNV incidence reports. The three regions utilized in this particular investigation were the Northern Great Plains (NGP), the Upper Midwest (UM) and the Southcentral states (SC). Those states considered part of the NGP were Minnesota, Nebraska, North Dakota and South Dakota. The UM states were Illinois, Indiana, Michigan, Ohio and Wisconsin, and the SC states were Arkansas, Louisiana, Oklahoma and Texas.

Additionally, the research highlighted the importance of precipitation by expressing that variable precipitation measures from three regions within the United States led to varying incidence of West Nile Virus (Wimberly, Lamsal, Giacomo & Chuang, 2014). Generally across the United States anomalies in temperature and precipitation have led to above average WNV incidence. This finding was reported in a study of neuroinvasive WNV incidence from 2004-2012 in ten different climate regions of the U.S. The authors of the study noted that above average yearly temperature was consistently associated with an increase in WNV cases. Less than average precipitation resulted in an increased WNV incidence in the Eastern regions and a decrease in case incidence in the Western regions (Hahn et al., 2015). Broad studies, either on a global scale or nationwide scale like those reported within this section, are crucial for researchers given the global impacts the illness has created. However, a few studies have emphasized the relevance of research at the city level. For instance, since WNV was discovered in New York in 1999, the United States has used the city of Houston, Texas as a surrogate model for the evolution of the virus (Mann et al., 2013). In Chicago, Illinois, Ruiz and colleagues focused their studies on modeling the factors that increase WNV incidence within the city.

Conclusions of the study generally followed what was known so far, adding to the growing body of literature on this topic. Researchers discovered that an increase in outdoor temperatures and a dry Spring season followed by a wet and rainy Summer in Chicago increased the WNV incidence between 2004 and 2008. Furthermore, the mathematical model proposed that the elevation of Chicago had a moderate influence on the incidence of the virus (Ruiz et al., 2010).

Related Works on Maricopa County

Among all of the literature works used as the basis for the current thesis, two articles explicitly discussed findings from the 2010 outbreak of WNV in Maricopa County, Arizona (Godsey et al., 2012 & Gibney et al., 2012). Once the alarming connection between mosquito populations and

WNV incidence in the early weeks of August became a priority for the area, the abundance of mosquito vectors was under investigation. Gravid traps were placed in 12 locations within the county. Six of the mosquito collection sites were in outbreak sites and the other six were in control sites where no WNV cases had been reported. Among the 12 sites, the authors kept the demographical characteristics of the population as close to identical as possible. When the results were compared from the two separate groups of traps, the investigators discovered that those traps from outbreak sites had at least twice the number of mosquitoes than the traps from non-outbreak sites.

The observed difference was attributed to a higher frequency of interaction between the vectors and human hosts in the outbreak areas. This was confirmed because those sites were the only sites to trap female *Culex quinquefasciatus* mosquitoes with evidence of having taken blood meals from humans (Godsey et al., 2012). Rather than studying mosquito populations, a human case-control study was performed during the 2010 outbreak in Maricopa County. The work was aimed at uncovering a connection between the location of residence and WNV. There were 49 patients and 74 controls involved. All of the subjects were interviewed, family members in the same household were surveyed, and environmental data from the area was collected. The adjusted odds of acquiring WNV among patients in the study who were served by Water District X within Gilbert Township was 5.2 times the adjusted odds of those residing in another water district. Individuals with standing water in containers in or around their yard or home had an adjusted odds of contracting WNV that was 5.0 times that of those who did not have any standing water around their homes. Finally, individuals who did not leave their home for work or school had an adjusted odds of acquiring WNV that was 2.4 times the adjusted odds ratio for individuals who leave their home for such activities (Gibney et al., 2012).

Author(s)	Year	Location	Important Points or Findings
Conte et al.	2015	Mediterranean Basin and Central Europe (43 countries)	-Studied the sustainability of ecological areas for transmission of WNV -Desert areas -Temperate areas
Chen, Epp, Jenkins, Waldner, Curry & Soos	2013	Canadian provinces of Alberta, Saskatchewan and Manitoba	-Authors examined 2005-2008 prevalence of WNV in *Culex tarsalis* -Higher infection rates and increased numbers of mosquitoes were correlated with high mean temperature - Increased temperature variation may play a role in limiting the midgut infection of WNV
Chen, Jenkins, Epp, Waldner, Curry & Soos	2013	Canadian provinces of Alberta, Saskatchewan and Manitoba	-Goal: forecast the spatial and temporal distribution of *Culex tarsalis* and WNV infection rate for climate conditions in the years 2020, 2050 and 2080 using a median situation and two extreme conditions -Where WNV is endemic, the median infection rate in 2050 increased by 17.91 times (compared to climate conditions in 2010) -The northern reach of mosquitoes doubled geographically
Chuang & Wimberly	2012	Northern Great Plains (NGP) of the U.S.	-Environmental model of WNV risk in the region -The model used satellite remote sensing data from 2004-2010 -These authors also looked at other factors including vegetation, land surface temperature and evapotranspiration
Morin & Comrie	2013	Regional study of the United States	-Main findings from this study focused on the southern U.S. -South: habitat drying and early death of the vector will decrease human case numbers in the future -Southwest: mosquito season is delayed due to higher temperatures in the spring and summer with rain in the late summer and fall
Hahn et al.	2015	United States-divided into ten climate regions	-This was a study of WNV neuroinvasive disease -The team found that incidence increases were associated with weather conditions -Anomalies in temperature and precipitation were associated with above average WNV incidence across the United States
Wimberly, Lamsal, Giacomo & Chuang	2014	Three regions of the US with the highest incidence in 2012: NGP, SC and UM	-Authors found that low (winter) temperatures were associated with fewer human cases -They noted a weak association of spring/summer temperatures with human case numbers -Variable precipitation across regions gave variable impacts on human case numbers

Ruiz et al.	2010	Chicago, Illinois 2004-2008	-Developed a mathematical model of the virus and found that elevation had a 'moderate' impact on incidence -Factors increasing WNV incidence included increased air temperature, a dry Spring season followed by a wet/rainy season, and low precipitation -Changes in weather patterns resulting from climate change need to be studied in more detail so predictions about WNV and other vector-borne disease risks can be as accurate as possible
Brown, Young, Lega, Andreadis, Schurich & Comrie	2015	Eight northeastern states	-Examined the relationship between human incidence and county urbanization which resulted in the discovery of a significant spatiotemporal pattern -Found that urbanization is a risk factor for WNV incidence based on county-level data from 1999-2006 -Counties with less than 38% forest cover had 4.4 times greater odds of WNV incidence being greater than 0.75 per 100,000 population
Gibney et al.	2012	East Valley of metropolitan Phoenix, Arizona	-A case-control study in Arizona in 2010 -Those individuals living in a certain water district who had standing water in their yard and did not leave the home regularly for work or school had an increased odds of contracting WNV compared to the controls -Artificial water sources are important to consider in terms of mosquito breeding
Godsey et al.	2012	Maricopa County, Arizona	-During 2010, mosquito traps in Maricopa County had more than twice the number of vectors in the traps -The team attributed the difference to a higher frequency of interactions between the vector and human hosts in the outbreak area -Human blood meals only found in *Culex* mosquitoes at outbreak sites

Table 1. Literature Review Summary

CHAPTER III: METHODOLOGY

3.1 Data Sources

For the intentions of this thesis, two main data sources were compiled. All of the data used for this study were secondary data and available to the public via the Internet. In this work, the author considered the relationship of West Nile virus incidence with the following four climatological metrics: minimum temperature (˚C), mean temperature (˚C), maximum temperature (˚C) and precipitation (mm).

Maricopa County, Arizona: West Nile Virus in Humans

Data regarding the WNV incidence in the selected county of Arizona for years 2007-2013 were obtained from the Maricopa County Department of Public Health. Their website is http://www.maricopa.gov/PublicHealth/Services/EPI/ and presents the annual data by week. For each week during the study period, the yearly epidemiologic surveillance data was used to obtain the total number of human cases. All of this data was then added to an aggregate spreadsheet for the study dataset. From 2007 through 2009, the population demographics were not published as part of the general epidemiological surveillance report. The vector surveillance system also changed, but not at the same point in time. In Arizona, the species of vectors found in a given county were reported during 2007 to 2010, but eliminated by the 2011 reports. Exclusion of this information added limitations to this study because demographics of the population and vector abundance were not available for annual comparisons. Figure 5 illustrates the overall WNV incidence presented by year for the county under investigation. For all purposes throughout this thesis, the term incidence refers to weekly case counts from the county level. Therefore, by week, the incidence data is technically the number of new cases occurring during that specific week. For this study, all of the surveillance data were available for half of the weeks of the given years.

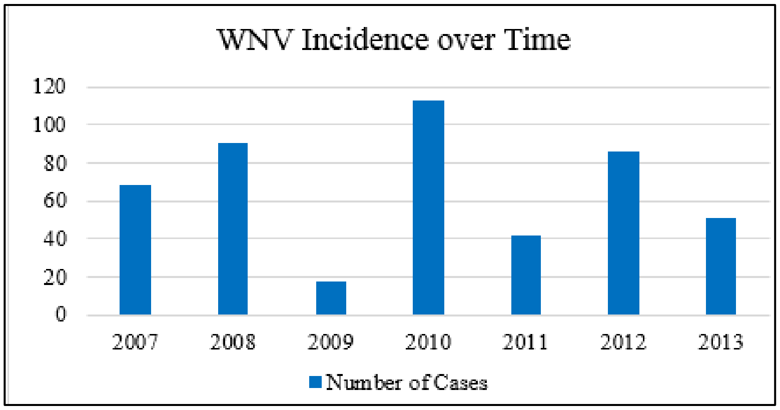

Figure 5. Incidence of West Nile Virus in Maricopa County, 2007-2013
Annual incidence of West Nile virus in Maricopa, County Arizona. The total number of cases during each year is shown. During the entire study period (2007-2013) there were 469 cases of West Nile in Maricopa County.

The general lack of WNV incidence data from mid-November through May of the next calendar year is a result of decreased surveillance during these months. Winter temperatures generally inhibit mosquitoes from breeding successfully, reducing the need for females to feed on susceptible hosts. Thus, zeros were recorded for the WNV incidence during the winter and spring seasons of the years under study. By the start of June, temperatures are high enough to support WNV transmission more efficiently. The case definition used by officials in Maricopa County, Arizona for West Nile Virus infection is the same as the case definition used by the CDC. A case of WNV is identified by clinical compatibility with the illness and laboratory confirmation.

Climate data

All of the data concerning maximum, mean and minimum temperatures and precipitation were obtained from the same source. This data was extracted from the United States Climate Data web page, which can be located at http://www.usclimatedata.com/climate/arizona/united-states/. Daily records were used to compute weekly averages where applicable. Only data that corresponds to the given years were included. Data was added to the study to increase the sample size because WNV is a rarely diagnosed disease and the sample size for each year in the study is small. The additional data was purely composed of climatic data and the incidence was assumed to be zero. This assumption reflects the lack of available case reports due to limited surveillance during the Winter and Spring seasons. However, due to changes in the reporting methods of the United States

Climate Data resource, the data that was added came from the Phoenix area reports rather than Maricopa County level data. Since Phoenix is located in Maricopa County, the climatic data would be interchangeable, thus justifying this change. Figure 6 exemplifies that the temperature measures have a pattern between them. This is shown by the simultaneous changes over time. Average low temperatures were mildly more variant but generally consistent.

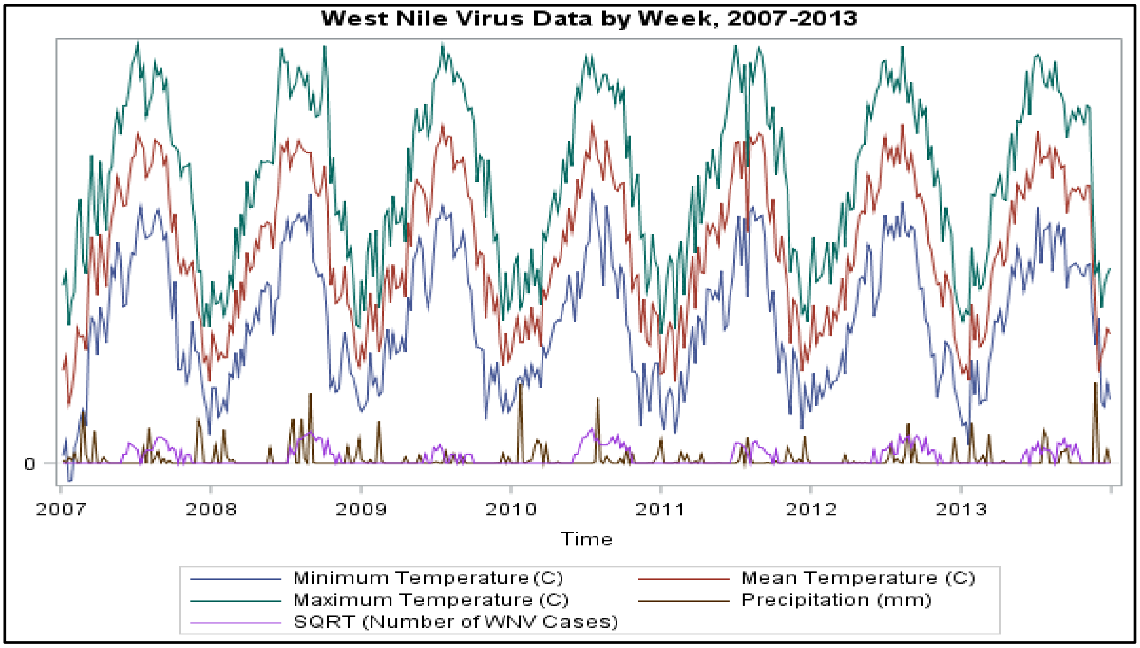

Figure 6. Time Series of Raw Data

The time series graph of the original dataset. A square root transformation was applied to the dependent variable to help stabilize the variance in the dataset.

Once the datasets were compiled together, the total number of cases was 469. Table 2 displays all of the summarized data including the total number of cases per year, the average maximum, mean and minimum temperatures each year, as well as the average annual precipitation. The generation of Figure 6, as well as the summary data in Table 2, prompted the statistical analysis rendered in the next chapter. Bivariate regression analyses were undertaken on each of the variables to determine how much of the variance was explained when all other climatic variables were removed.

Year	Maximum Temperature (◦C)	Mean Temperature (◦C)	Minimum Temperature (◦C)	Precipitation (mm)
2007	31.67	23.33	15.09	0.58

2008	31.06	23.78	15.75	0.67
2009	31.52	23.57	15.62	0.23
2010	30.79	23.03	15.28	0.59
2011	30.61	22.70	14.78	0.37
2012	32.06	24.08	16.10	0.29
2013	32.27	24.56	17.20	0.60
Overall	31.43	23.58	15.69	0.48

Table 2. Summary of the Variables of Interest over Time

3.2 Study Setting

The population under study is the entire population of Maricopa County, Arizona throughout 2007 and 2013. Figure 7 demonstrates the location of the county under investigation within the state shaded in dark blue. This county is located in the southwest region of the state of Arizona. Southern Maricopa County lies within the Sonora Desert. The county encompasses the capital city of Phoenix, Arizona and covers 14,846 kilometers of land. The average rainfall per year is about 203 millimeters, while the national average rainfall is about 940 millimeters. Annually, Maricopa County experiences zero millimeters of snowfall, while the national average is 635 millimeters. The county sits 0.3 meters (or 1,160 feet) above sea level. The north, east and west sections of the county are surrounded by mountain ranges. Daily mean temperatures in this area hover around 21.8˚C. Five rivers flow through the county, including the Salt, Gila, Hassayampa, Agua Fria and Verde Rivers. This county is also home to five Indian reservations and part of the Tonto National Forest. During the study period, the average population of the area was approximately 3.9 million individuals. The population density is about 415 individuals per square mile. Population demographics for those with West Nile infections were excluded because the data were not available for all of the years.

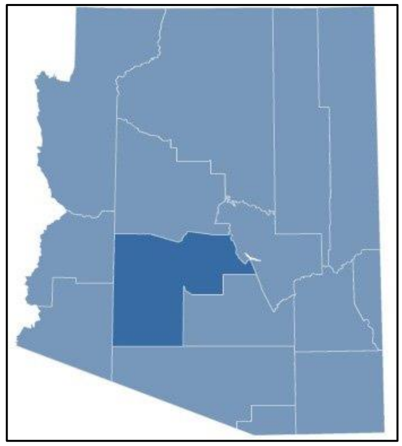

Figure 7. Geographical Location of Maricopa County
Map of the state of Arizona with all counties outlined. Maricopa County is located in the southwestern region of the state and is shaded dark blue. It covers 14,846 kilometers of land and sits 0.3 meters above sea level. The county has a temperate climate and a population of roughly 3.9 million individuals.

Each week number corresponds to the given date of the end of that week on the calendar. For example, Week 23 falls on the second week of June in 2007 and Week 304 falls on the last week of October in 2013. These variables were in the datasets for the majority of the period, limiting much of the need to account for missing data. Specific to this study, the missing data was addressed and coded to fit the study objectives as closely as possible. For continuous variables (temperature and precipitation), there was only one observation that was missing data. The overall seven-year average was used to account for the missing data. After coding for missing data was complete, the remaining variables were coded in the same manner the variable was reported in the public data unless categorizing the variable was shown to be more beneficial. For instance, outliers were identified easily when the number of cases per week was categorized into six levels by temporarily grouping similar observations into the same categories. All calendar dates were left in the dataset, but the time component was removed because it was an irrelevant factor.

CHAPTER IV: RESULTS

4.1 Yearly Discussion of the Data

This section of the chapter will include a discussion of the original data before any analysis was conducted. During the year of 2007, 68 individuals became symptomatically ill from West Nile Virus. The amount of rainfall that was seen during this time was generally low, but did reach a peak in early August. Interestingly, the cases peaked about three weeks later. Given the knowledge in the field surrounding rainfall and mosquito breeding periods, the potential relationship observed here was kept in mind for further investigation. The year of 2009 also presented a peak in the rainfall in August, prompting the need for analysis. Across the entire study period, 2010 was the year with the highest WNV incidence, partially due to the declared outbreak that year. This outbreak was the second largest outbreak seen in the history of Maricopa County (Komar, Panella, Young, Brault, & Levy, 2013). There was a total of over 100 cases, and nine infections led to fatalities. Incidence peaked in mid-July, during which there was no measurable precipitation in the county. In 2011, about 70 percent of the cases occurred in July or August, which falls within the peak of WNV season, as defined by the CDC. During 2012, the peak rainfall was observed in August once again. Incidence peaked multiple times across August and September, potentially continuing the trend noted in some of the other years.

4.2 Statistical Analysis

The analysis, conducted on a Windows 7 machine using SAS 9.4, was carried out to determine the impact of minimum temperatures, mean temperatures, maximum temperatures and precipitation on the incidence of West Nile Virus (spanning 2007 to 2013). Based only on the time series graph of the raw data, there is a clear visual pattern between temperature measures and incidence. However, before any conclusions could be drawn, the analysis had to be conducted. At the beginning of the analysis, the square root of the WNV incidence was applied to assist in stabilization of the variance. Next, the correlations among the variables themselves were checked against the WNV incidence. Those variables with the highest correlation coefficients were maximum temperature (r=0.65) and mean temperature (r=0.65), followed by minimum temperature (r=0.62) and precipitation (r=0.09). The baseline data prior to any modifications or regression analysis resulted in the following model: SQRT(WNV Incidence)=-3.19386 + 0.17197 (precipitation) + 0.06898 (maximum temperature) + 0.00783 (mean temperature)+ 0.08250 (minimum temperature).

Multiple linear regression was performed next using the four climatic variables as predictors and WNV incidence as the outcome with a significance level set at 0.05, leading to a 95 percent confidence level. When all of the variables were included in the model, only precipitation had a statistically significant association with the outcome of interest. The full regression model is illustrated by the following model: SQRT (WNV Incidence) =-1.59458 + 0.12669 (precipitation) +0.06314 (maximum temperature) -0.00148 (mean temperature) +0.01264 (minimum temperature), which explains about 89.93 percent of the observed variance with a p-value <0.0001. A stepwise multiple linear regression model was also generated. This analysis explained 87.95 percent of the observed variance in the data with a p-value of <0.0001. The overall stepwise regression analysis is given by the following model: SQRT (WNV Incidence) =-1.69001 + 0.07124 (maximum temperature)+0.13555 (precipitation). Each of these predictor variables was significant at the 95 percent confidence level. Figure 8 demonstrates each of these models graphed over the entire study period.

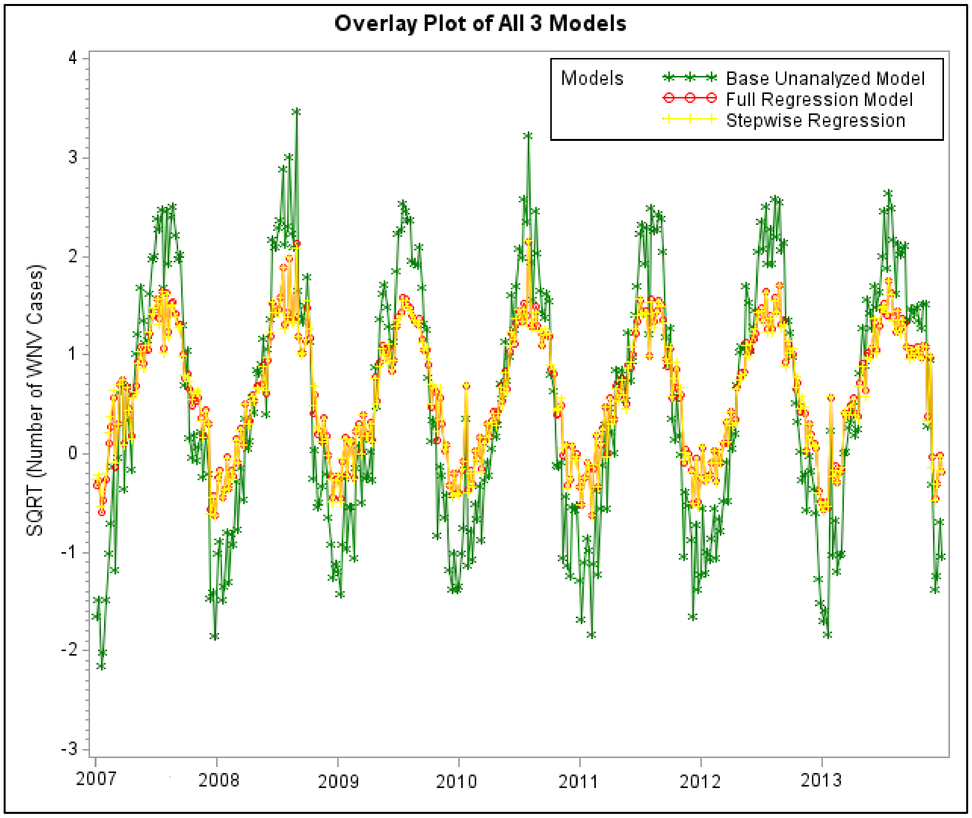

Figure 8. SQRT Transformation of the models fitted to the data

Illustration of the three models used in the study; The green line represents the entire raw dataset before any regression approaches were applied to the data. Multiple linear regression was utilized to produce the fit of the full regression model, shown in red. This model explained 89.93 percent of the variance observed in the original data. The yellow line depicts the fit of the stepwise multiple linear regression model, which explained 87.95 percent of the variance seen in the original data.

CHAPTER V: DISCUSSION AND CONCLUSION

5.1 Discussion

This work has presented an assessment of four climatic variables as predictors of West Nile Virus incidence. All of the data were from 2007 through 2013 and originated from Maricopa County, Arizona. Temperature variables were discovered to be significantly correlated with the number of WNV cases. Despite this fact, only the maximum temperature measure was statistically associated with the incidence of WNV. This suggests that the other two temperature measures may be confounding the relationship between incidence and precipitation. In fact, precipitation was found to hold a statistically significant role in the present study as well. These findings align with the work of Deichmeister and Telang (2011) where it was found that high temperatures and low amounts of precipitation were valid predictors of the number of mosquitoes. Another study noted that areas experiencing a drought have conditions that promote WNV spread. This increased spreading is due to the growing interaction between the mosquito vector of the area and the native, reservoir birds (Montgomery & Murray, 2015).

Given that the data for the present study covered a total of 365 weeks across seven years, the findings were not surprising. The analysis revealed that high temperatures and low amounts of rainfall result in increased WNV incidence, suggesting that the null hypothesis should be rejected. The two models that were generated from the data were only capable of explaining about 45 percent of the observed data. This implies that there may be other factors involved. Factors like land surface temperature, vegetative growth, canopy cover, wetland coverage, humidity and numerous other aspects of the outdoor environment are potentially confounding the relationships that were observed. Such factors have been measured in other works (Brown, Young, Lega, Andreadis, Schurich & Comrie, 2015; Deichmeister & Telang, 2011; Chuang & Wimberly, 2012), but access to such information was not readily available for all of the years included in this work.

5.2 Study Limitations

The present study was able to meet the defined objective and answer the research questions. With that in mind, this thesis is not without its flaws. For instance, one must consider that in general, only one out of every five people, or 20 percent, develop symptoms of WNV infection. Thus, the observed incidence is also underestimated in this respect. In addition, the work completed in this thesis only accounted for about 45 percent of the variance seen in the raw data. Inferences about confounding can be drawn from this conclusion. If temperature and rainfall measures only explain 45 percent of the variance, there must be other factors involved in the transmission mechanism that were not included, such as those mentioned in the previous section. Another important limitation is introduced by the seasonality of WNV surveillance. The incidence data in the study is only included for the CDC defined West Nile Virus season, which ranges from June to about mid-November of each year. There is little to no surveillance during the other six months of the year. Due to this fact, the incidence measure in this work was set to zero for these six months. It is possible that this data imputation skewed the results.

Since the available data was only accessible for the Summer and Fall seasons, there may be other seasonal influences on the incidence that simply are not observed in this work. In addition, the cases that may or may not take place during the Winter and Spring are not included at all. This fact is potentially leading to a vastly underestimated incidence for the seven year period. Implementing a surveillance program that is active year-round could assist in decreasing the scope of this underestimation. Continuous surveillance is generally a costly endeavor for counties. Statewide surveillance is also expensive, but states would have more resources available to them than would an individual county. Implementing a continuous surveillance system within the state would help close the gap and better account for the true WNV incidence published in public reports. Federal funds for WNV investigation should supplement the efforts put forth by the state, but there will always be differences at federal and state levels.

5.3 Recommendations and Conclusion

For this study to be improved, the research question should be narrowed to study either temperature or precipitation impacts on West Nile Virus incidence over time in Maricopa County, Arizona. By selecting only one of these main impacts on incidence, other variables can be added to explore the relationships. Continuous surveillance and monitoring of mosquito populations are both essential for mosquito control and WNV prevention. Despite the cut seen in federal funding set aside for WNV surveillance in 2012 (Hahn et al., 2015), several states still have ongoing surveillance programs. This surveillance is state funded, but the findings are not reported to the public unless it is deemed necessary as defined by the given state. Similar to reporting these findings, it is also important to understand how the climate influences viral transmission. For example, researchers need to include climatic factors in mechanistic models of WNV transmission dynamics, as seen in other studies (Brown, Childs, Diuk-Wasser & Fish, 2008). By successfully establishing a transmission model of West Nile virus, statewide or even nationwide public health officials and interventionists can begin to develop strategies to mitigate the spread of the virus across multiple geographical locations with varying circumstances.

Future studies could include factors like mosquito density, human development indices, urbanicity or other factors to measure how the climate, mosquitoes, and humans all interact. This would allow for the development of one overall understanding of WNV transmission and multiple factors that may change incidence in a given area or region of the world. With current climate change discussions going on globally, WNV will remain a problem for those in public health for quite a long time. The climate is one of many aspects of the environment that influence WNV transmission. By incorporating local climate conditions into transmission models and disease forecasting, the public health community can develop more effective interventions ahead of the outbreak conditions. Adding effective interventions and an educated general population together is the best way to move forward and limit the spread of this viral illness locally, regionally, and even globally in the near future.

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