

**A GLOBAL APPROACH TO DISEASE PREVENTION: PREDICTING  
HIGH RISK AREAS FOR WEST NILE INFECTION IN THE US**

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## **ABSTRACT**

First identified in 1937, West Nile (WN) virus has affected birds, equine, and humans throughout Africa, Asia, and Europe. Its 1999 appearance in New York shows the ability of the virus to cross barriers and travel great distances emerging into new territories previously free of infection; and spreading much faster than expected, WN virus has infected thousands of birds, equine, and humans throughout the conterminous United States (US). Case and serological studies performed in the Eastern hemisphere prior to 1999 offer detailed descriptions of endemic and epidemic locations in regards to geography, land cover, land use, population, climate, and weather patterns. The patterns and values associated with these environmental factors associated with WN virus activity can identify the landscape of the disease and contribute to the development of a model of risk to identify US counties of high risk for WN infection. State and county public health officials can use this model as a decision making tool to allocate funding for disease prevention and control. Dynamic factors associated with increased transmission, such as above average temperature and precipitation, can be closely monitored and measures of prevention can be implemented when necessary. In turn, detailed information from higher resolution analyses can be documented to an online GIS (Geographic Information System) that would contribute to a global collaboration on outbreaks and prevention of disease.

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# **A GLOBAL APPROACH TO DISEASE PREVENTION: PREDICTING HIGH RISK AREAS FOR WEST NILE INFECTION IN THE US**

## **CHAPTER 1: INTRODUCTION**

Emerging infectious diseases (EIDs), as defined by Morse and Schluederberg (1990), include diseases new to a population, or that have recently increased in incidence or geographic range. These diseases are a global threat; with the right conditions, a newly emerging infection could spread throughout an entire continent within a few days (Morse 1995). For example, the emergence of a new virus subtype caused the Spanish influenza pandemic of 1918; within one year, this global outbreak revealed the power of an EID by claiming the lives of 20 million people, and over 500 thousand within the United States (US) (Simonsen, Clarke et al. 1998). In the 1980s, acquired immune deficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), emerged and spread quickly throughout the US (Gould 1993). The CDC (2009) estimates 1,051,875 cases and 583,298 deaths have occurred since the epidemic began.

Between the 17<sup>th</sup> and early 20<sup>th</sup> centuries, vector-borne diseases were responsible for more human deaths than all other causes combined (Gubler 1991). Making up nearly 80 percent of known animal species and including ticks, fleas, flies, and mosquitoes, arthropods are considered the most significant vectors of disease (Gubler 2001; Black IV and Kondratieff 2005). The most important arthropod vector is the mosquito. With 3,500 different species and subspecies, mosquitoes are highly abundant and able to adapt to nearly every type of habitat (Eldridge 2005). They are found on every continent except Antarctica, wherever standing water exists, and at a range of elevations from below sea-level to over 3,000 meters, mosquitoes have the ability to adapt to nearly every type of habitat (Eldridge 2005). Mosquito eradication

programs have failed in the past and the diseases they spread continue to have a large burden on human health. Over 500 million human cases and 1.5 to 3 million deaths occur each year from mosquito-borne diseases, such as malaria, dengue, and West Nile (WN) virus (Campbell, Marfin et al. 2002; World Health Organization 2002).

WN virus was identified in 1937 from the blood of a woman living in the West Nile district of Uganda (Smithburn, Hughes et al. 1940). According to the CDC (Staples 2009), the distribution of WN virus up until 1998 was very broad throughout the Eastern hemisphere. However, sixty years of outbreaks and serological studies offer detailed descriptions of endemic and epidemic areas within Africa, Europe, and Southeast Asia, limiting WN virus activity to a much smaller area than argued by the CDC (Campbell, Marfin et al. 2002). Incidence of WN virus among endemic regions in the Eastern hemisphere shows the ability of the pathogen to sustain itself within an ecosystem for a long period of time. The 1999 emergence of WN virus within the Western hemisphere confirms exotic infectious diseases are capable of crossing great distances to affect new territories that were previously free from its disease (Campbell, Marfin et al. 2002).

WN virus has since spread throughout the conterminous US, and among areas in Canada, Mexico, Central and South America, and the Caribbean (Komar and Clark 2006). With ten years of consistent seasonal activity, WN virus has become the number one cause of neuroinvasive disease among humans (Staples 2009). Approximately 11,772 cases of severe WN neuroinvasive disease (WNND) and 1,129 deaths have been reported thus far (United States Geological Survey 2007; Centers for Disease Control and Prevention 2009), and according to the Centers for Disease Control and Prevention (CDC), estimates suggest 1.65 million people have likely been infected (Staples 2009).

## 1.1 PROBLEM STATEMENT

Globalization, climate change, shifting weather patterns, pathogenic evolution, drug and insecticide resistance, increased vector habitat and populations, and the lack of preventative measures have eliminated barriers and encouraged the emergence and reemergence of vector-borne diseases throughout the world (Ozer 2005). Easier and faster means of transportation are encouraging people to travel more frequently and at greater distances, and as the demand for international travel continues to rise, exposure to new and different strains of infectious agents is becoming more frequent, escalating the risk of human infection and providing a global route of disease transport (National Institutes of Health and National Institute of Allergy and Infectious Diseases 2006). Given the impact mosquito-borne EIDs can have on the health of human and animal populations; there is a need to study and predict the distribution high risk areas for the prevention and control of future epidemics. Early prediction is essential to allow public health officials to educate the population and prepare for the prevention of major outbreaks before they occur.

Since the end of mosquito eradication programs in the 1970's, the focus of US health care shifted from prevention to emergency response (Staples 2009). The arrival of WN virus to New York in understandably a shock; however, approximately four years passed before the US experienced the greatest impact of the disease (United States Geological Survey 2007). Approximately 10,000 cases of WN infection and 3,000 cases of WNND reported among humans in 2003 (United States Geological Survey 2007). Given 60 years of research and 4 years of WN activity in the US, could US public health officials have been more prepared to prevent or control the spread of WN infection?

Based on the theoretical context of disease ecology, human ecology, landscape epidemiology, and spatial epidemiology, there are two basic purposes of this study:

- (1) Complete a fine-scale analysis of environments where WN virus activity has been reported in the Eastern hemisphere (study areas) to identify the important factors and processes that define and influence the distribution of human risk for WN infection.
- (2) To develop a static model of risk based on the range of variability of each factor among the study areas to predict US counties that are high-risk areas for increased transmission of WN virus and incidence of human WNND.

The applied purpose of this study is:

- (3) To standardize a method that can be applied to predicting the risk of other vector-borne diseases in preparation to improve the rate and efficiency of their prevention and control.

In this study, the term “environment” is broadly defined and refers to the total surroundings of the natural and built environment, which are associated with the increased transmission of WN virus. Environmental factors considered in this model include including elevation, land cover, vegetation, and climate. Using a Geographic Information System (GIS), environmental data will be evaluated among 25 study zones with reported WN activity in the Eastern hemisphere. The objective of these analyses is to identify the patterns and values of each factor associated with different levels of risk based on the severity of WN activity within each area. The purpose of this research is to create and validate a geographic model of risk for WN infection in the US. The model will be based on the range of variables that are associated with each environmental factor among the study areas. The accuracy of this model will be compared with the cumulative incidence of WNND from 2001 through 2008.

## **1.2 SIGNIFICANCE OF STUDY**

Analyzing the environment within the actual boundaries of well documented WN virus activity in the Eastern hemisphere contributes to a more fine-scale understanding about the distribution of WN virus and the values of each variable that are associated with different levels of outbreak risk. Several studies have looked at specific influences such as temperature, precipitation, and the distribution of dead birds and human incidence, but none have outline all of the factors and processes that may affect the transmission of WN virus. In addition, most studies have focused on a small-scale area where WN virus activity has been reported. Although the information obtained from these studies is relevant to the validation of important factors, they do not consider the global influences that may be involve. To predict disease risk at the national level, a broader approach is needed to capture all landscapes associated with WN virus activity.

In the fight to prevent the spread of other EIDs in the US, state and county public health officials can use this predictive model of risk as a decision-making tool to allocate funding for disease research, prevention, and control. Dynamic factors associated with increased transmission, such as above average temperature and precipitation, can be closely monitored and measures of prevention can be implemented when necessary. In turn, detailed information from higher resolution analyses can be documented to an online GIS that would contribute to a global collaboration on outbreaks and prevention of disease.

## **CHAPTER 2: LITERATURE REVIEW**

### **2.1 INTRODUCTION**

This review will examine the current body of literature pertaining to theoretical approaches and methods within medical geography and current studies modeling disease risk and prediction. Background information on WN virus vectors, hosts, and disease will provide insight on the dynamics of its transmission. Based on the surveillance of mosquitoes, birds, horses, and humans, a summary of WN virus activity from 1999 to 2009 will demonstrate the 10-year continuous impact of infection within the US. The history of epidemics and serological studies performed in the Eastern hemisphere prior to 1999 will be evaluated to identify the patterns associated with three levels of risk and the values of environmental factors that support or sustain WN infection and contribute to its spread.

### **2.2 THEORIES IN MEDICAL GEOGRAPHY**

Medical geography integrates geographical methods and techniques with perspectives from multiple scientific disciplines to study health-related issues (World Health Organization 2002). Those who focus on the social aspects of health and health care prefer to be known as health geographers, whereas medical geographers are highly involved in the medical sciences and tend to focus on the spatial and temporal distribution of diseases (Rosenberg 1998; Meade and Earickson 2000; Gesler 2004). Pathology, ecology, epidemiology, and geomedicine are just a few of the many disciplines that have contributed to the methodology of the medical geographer (Rosenberg 1998).

This research will combine four theoretical approaches to determine the significant elements and processes that sustain WN virus within an ecosystem and increase the risk for infection among humans. The *Disease Ecology* approach examines the relationships between habitat populations within a changing ecosystem. *The Triangle of Human Ecology* is a holistic approach that focuses on how habitat, population, and behavioral relationships among interacting populations affect the human state of health (Meade and Earickson 2000). *Landscape Epidemiology* is an in-depth approach used to determine the arrangement of environmental components and patterns that support disease transmission. *Spatial Epidemiology* is a visual approach to assess and predict risk over time and space, incorporating three methodologies within a GIS: spatial distribution mapping, static risk mapping, and the incorporation of landscape elements. Together, these approaches will contribute to a better understanding about the dynamic transmission of WN virus and improve the ability to predict the emergence and outbreaks of its disease.

### **2.2.1 DISEASE ECOLOGY**

Disease ecology examines the relationships between populations and the changing environment, and studies how processes of population interactions support or discourage disease (Meade and Earickson 2000). In theory, the incidence, distribution, and timing of outbreaks can identify the spatial interactions among populations within the environment. The dynamics of disease transmission are defined by the changing overlap of habitats in time and space. Figure 2-1 shows the changing ecology of a vector-borne disease in non-epidemic versus epidemic environmental conditions. The non-epidemic environment has no overlap between the vectors and humans. The overlap in the endemic environment (dark blue), represents areas of risk for

human infection. Seasonal epidemics can be measured by the variable amount of the overlap over time and space.

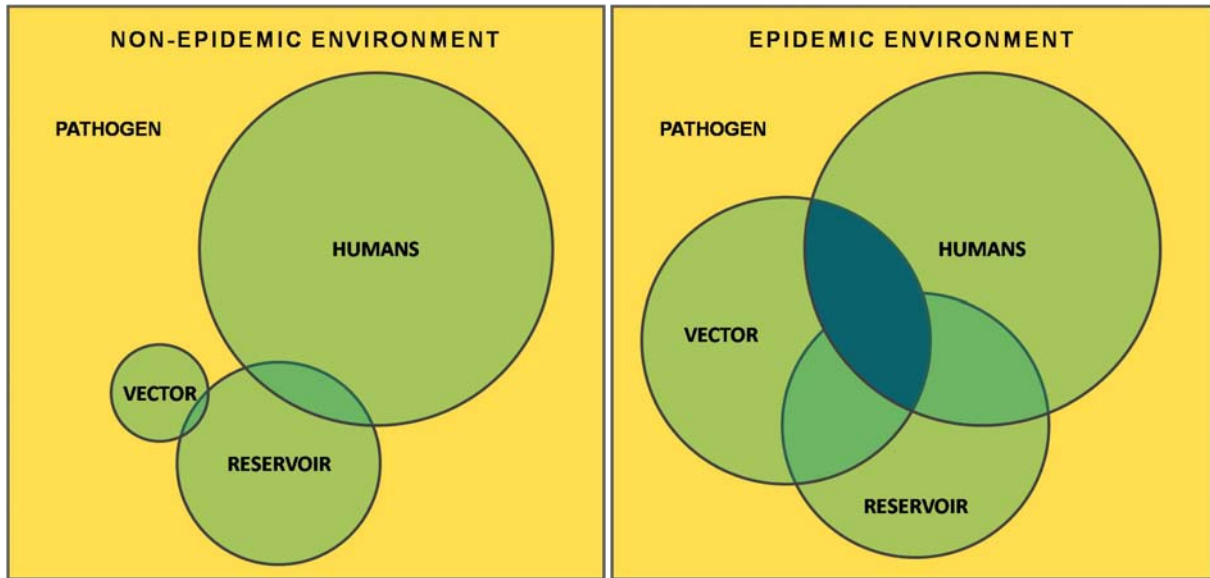


Figure 2-1: The ecology of vector-borne diseases.

As described by Meade and Earickson (2000), the triangle of human ecology (figure 2-2) focuses on three main categories of factors that affect the state of health: population, behavior, and habitat. The elements and processes that contribute to the ecology of an epidemic environment can be identified by the relationships and patterns among the interacting populations.

*Population* is any biological organism that may carry and host disease. Genetic susceptibility, age, and gender are some of the unique attributes that determine whether or not a host can physically and

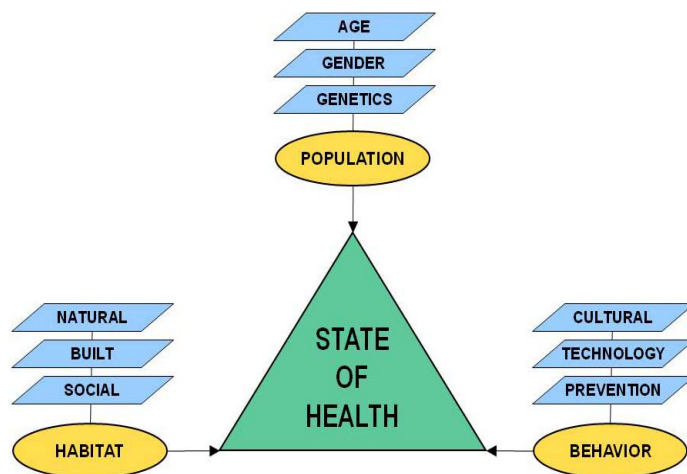


Figure 2-2: Triangle of Human Ecology.  
Adapted from: (Meade and Earickson 2000)



emotionally cope with infection (Meade and Earickson 2000). *Behavior* refers to the observed activities among a population, including cultural aspects, patterns of movement and migration, and the implementation of disease prevention and control. *Habitat* is divided into three sub-categories: the natural, social, and built habitats. *Natural habitat* refers to the topography, land cover, land use, climate, and weather patterns of a given area. *Social habitat* relates to family, friends, cultural, and spiritual influences. *Built habitat* includes all of the characteristics within, around, and between the buildings we live in, work at, and travel within during our daily activities; this incorporates all aspects of construction materials, sanitation and waste disposal, water sources, building design, air flow and lighting, health care facilities, and transportation (Meade and Earickson 2000). Differing from sociological models, the triangle of human ecology considers population and behavior as separate influences, and this approach is the basis for many studies questioning the arrival and spread of disease among humans (Gesler 2004).

For this study, the ecological triangle will be applied to the mosquito and human as separate entities. The patterns and associations between the important elements and processes that interact between the mosquito and human will identify human risk by the changes in the overlapping ecological habitat.

### **2.2.2 LANDSCAPE EPIDEMIOLOGY**

The landscape of a place is characterized by a mosaic of habitats within the ecosystem. The elements and patterns associated with the biotic, abiotic, and cultural processes within a landscape are used to identify the factors that influence disease transmission over time and space (Meade and Earickson 2000). Considering the elements and processes, or the factors that encourage or suspend the spread of disease, this approach has been used to define the landscape epidemiology, or geographic boundaries and pathways of transmission, for diseases such as the

plague, malaria, dengue, and schistosomiasis (Meade and Earickson 2000). Public health officials evaluate the epidemiological pathways to determine if landscape modifications can be used to block the route of transmission of a pathogen for the prevention or control of its disease (Meade and Earickson 2000).

There are three basic observations that define the concept of landscape epidemiology: first, the geographic distributions of diseases tend to be limited; second, variations in physical and biological factors that support the virus, vectors, and reservoirs may cause spatial restrictions or barriers; third, if the conditions of the supporting factors can be captured on maps, then the current and future risk should be predictable (Meade and Earickson 2000). The important environmental factors are determined from the examination of the biome and natural nidus of a region where disease activity exists. Identified by its distinct plants, animals, insects, and microbes, a biome is a broad biotic region that is highly predictable based on climate, altitude, and latitude. The natural nidus is the habitat composed of the vectors, hosts, and reservoirs involved in a continuous cycle of transmission that sustain a pathogen within an ecosystem (Ostfeld, Glass et al. 2005).

Based on outbreak reports and serological studies performed in endemic and epidemic regions, environmental variable values can be associated with the distribution of WN virus and the level of risk for infection (Ostfeld, Glass et al. 2005). The landscape epidemiology approach will be applied to determine how environmental variability affects the distribution of disease vectors, the rate of transmission, and the level of risk for human infection. By evaluating the biogeography of the vector and host life cycles, the distribution and the factors associated with transmission of diseases can be determined. Temperature, precipitation, topography, land use,

and land cover, will be carefully reviewed to identify the factors and possible barriers that affect the transmission of disease under changing environmental conditions.

### **2.2.3 SPATIAL EPIDEMIOLOGY**

Given that the risk of contracting an infectious disease generally decreases exponentially with increasing distance from an infected host, the factors and processes that affect the spatial distribution of the pathogen, vectors, and hosts are essentially important for transmission to occur (Ostfeld, Glass et al. 2005). Using a GIS, spatial epidemiology examines the variation in the amount of risk or incidence of a specific disease over time by incorporating case data with habitat modeling techniques (Meade and Earickson 2000; Ostfeld, Glass et al. 2005).

Spatial distribution mapping requires precise spatial and temporal data on disease incidence to target the areas at risk for the implementation of preventative measures within a timely manner (Ostfeld, Glass et al. 2005). This technique will be used to evaluate the existence and the spread of WN virus in the Eastern hemisphere as well as its establishment within the US. Static risk mapping considers disease incidence with vector, reservoir, and host distributions to determine the ecological risk of exposure to an infectious disease (Cummings, Irizarry et al. 2004). Based on disease ecology, the overlay of habitats will identify the level of ecological risk for human exposure. Outlined in the previous section on landscape epidemiology, the incorporation of biotic and abiotic elements and processes will identify the level of risk for an epidemic WN infection based on changing environmental conditions.

## **2.3 DISEASE RISK MODELING**

Several mathematical models have been developed to investigate the host-vector transmission dynamics of WN virus; while these may provide important insights to the biological

factors of the disease, they do not explore the distribution of environmental components, and therefore will not be reviewed in detail (Cruz-Pacheco, Esteva et al. 2005; Kenkre, Parmenter et al. 2005; Wonham, Lewis et al. 2006).

Spatial models are used to better understand the geographic risk of disease by identifying the patterns among the factors and processes that affect the transmission and spread of disease. Studies within North America have produced spatial and temporal models predicting disease risk based on the relationships between environmental, population, and surveillance data; including the distribution of mosquito species, collected dead birds, and reported cases of human and equine infection. Based on different approaches, methods, and combinations of supporting factors, each model has a unique design as described in the summaries below.

### **VEGETATION**

Brownstein et al. (2002) developed a model based on vegetation and human population to determine where the introduction of WN virus occurred in New York during the 1999 outbreak. They found that levels of the Normalized Difference Vegetation Index (NDVI) calculated from image data could be used as an indicator of vector mosquito habitat in urban areas ( $<0.2$ ). Ward et al. (2002) further examined the relationship between vegetation cover, based on NDVI value, and the spatial distribution of equine cases. They confirmed that a high NDVI value ( $>0.5$ ) was representative of suitable vector habitat in rural areas. They felt the NDVI was highly influenced by agricultural areas and would be of better use as vegetation declines with crop harvesting or as the fall season begins.

This study focused on how measures of vegetation can be used to identify vector habitat. The results from this study are important and can be used in future risk model development; however, this is not a holistic approach. Because of the complexity of vector-borne disease

transmission, considering only one element would not be representative of the actual habitat.

### **POPULATION DISTRIBUTIONS**

Tachiiri et al. (2006) developed a model to predict where the introduction of WN virus might occur in British Columbia. Static risk mapping singled out high-risk areas based on three characteristics: mosquito estimates based on average temperature, the quantity of high-risk birds, and total residents over the age of 60. As acknowledged by the authors, their model accuracy is questionable for several reasons: they had very little information on the biology and behavioral patterns of the local mosquitoes; they neglected to consider the affect of precipitation on mosquito population; and other factors such as vegetation, geology, housing density, or land use, were not considered.

Allen and Wong (2006) combined population factors based on mosquito surveillance, dead bird density, and reported human cases to model high risk areas for WN virus activity in the US. The objective of this research was to examine approaches that may reveal spatial patterns to help guide public health officials in determining the necessary actions for prevention and control. Two risk models were created based on different approaches: the first model evaluated vulnerable human population density with mosquito abundance, and the second model evaluated vulnerable population density and dead bird density. The results were very different, but this could be due to data uncertainties; the abundance of infected birds and mosquitoes was estimated from field samples, and the human case locations were generalized because of confidentiality issues. The authors acknowledged that their models were limited; it was not their intention to identify and incorporate the environmental factors that support the spread of the disease.

Theophilides et al. (2005) implemented the Dynamic Continuous-Area Space-Time (DYCAST) system, which is based on Knox statistical tests that examine the closeness in space

and time between dead birds and human cases of WN virus. Calibrated from the previous year, they were able to predict WN infection among five out of seven human cases, thirteen days before onset in 2001; however, this model does not consider mosquito habitat, environmental conditions, populations at risk, or other feedback from surveillance programs. All three of these studies focused identifying the spatial and temporal population boundaries associated with higher risk. Although this approach is important to determining increased risk based on habitat, the fact that environmental conditions and processes were not considered is very limiting to these models, and none of them could be representative of actual risk.

### **CHANGING WEATHER PATTERNS**

Shaman et al. (2006) examined the spatial and temporal correlations between land surface wetness, human cases of WN virus, and transmission to sentinel chickens in Florida. They found that drought forces the *Culex nigripalpus* mosquito into close contact with avian species, amplifying the number of infected mosquitoes. When the rain returns, the infected mosquitoes and birds scatter, spreading the virus to surrounding areas.

Ward (2005) looked at the association between local temperatures and cases of WN virus encephalomyelitis primarily among Amish-owned horses in Indiana. He found that risk was always greater in areas closest to Lake Michigan because of higher early-summer temperatures. This confirms results from Dome et al. (2005) that suggest that higher temperatures increase the likelihood of *Cx. pipiens* to become infected with WN virus and accelerates the distribution of the infection throughout its body.

Both of these studies identified the affects of changing weather conditions upon risk; however, the static landscapes of disease, or all of the environmental factors that exist in

transmission areas, were not incorporated. The results from these studies need to be applied as the processes that bring change to the static landscape of disease.

## **2.4 WEST NILE VIRUS**

The spread of WN virus in the US is the latest example showing the ability of an exotic virus to emerge into new territory and establish itself as a seasonal threat, impacting human and animal populations with thousands of infections. This section will provide background information about WN virus. The following topics will be discussed in detail: virology, vectors, reservoirs, hosts, transmission, clinical features and management, and prevention.

### **2.4.1 VIROLOGY**

WN virus belongs to the genus *Flavivirus* (Fauquet 2005). Typically spread by an arthropod vector, *Flavivirus* is the largest of three genera in the family *Flaviviridae* (White 1994; Fauquet 2005). Over 70 recognized members of this family (White 1994) have been divided into eight antigenic complexes based on serological and genetic factors (Calisher, Karabatsos et al. 1989). Typically spread by a mosquito vector, WN virus is one of ten serotypes among the Japanese Encephalitis complex, which contains some of the most important viral pathogens of the developing world (White 1994).

With proper magnification, the WN virus looks similar to a “bumpy gum ball” (Associated Press 2003). It is spherical in shape, with a diameter of approximately 50 nanometers (White 1994). Highly infectious, the genome contains positive single-stranded RNA (ssRNA); RNA viruses are known for having frequent mutations and a high rate of evolution (Holland, Spindler et al. 1982), making the evolution of future strains of WN virus a significant threat. The virus has already evolved genetically into two distinct lineages (Lanciotti, Ebel et al.

2002). Associated with severe outbreaks of WN ND (Petersen and Roehrig 2001), lineage 1 is composed of the Australian KUN and WN virus isolates from North, West, and central Africa; southern and eastern Europe; India; the Middle East; and North America (Berthet, Zeller et al. 1997; Scherret, Mackenzie et al. 2001). Circulate among enzootic cycles in West, Central, and East Africa, and Madagascar, isolates in lineage 2 have not been associated with severe human infection (Petersen and Roehrig 2001).

#### **2.4.2 VECTORS**

WN virus has been Isolated from 43 different species throughout the world and 42 within the US. Able to transfer enough of the virus within a blood meal to infect several species of birds and mammals, mosquitoes are the dominant vectors of WN virus (Hubalek and Halouzka 1999). Largely ornithophilic, or bird-feeding mosquitoes, and also known to feed on mammals, the most competent vectors are members of the *Culex* species; specifically *Cx. univittatus*, *Cx. pipiens*, *Cx. tarsalis*, and *Cx. restuans* (Taylor, Work et al. 1956; Jupp 1974; Hubalek and Halouzka 1999; Turell, O'Guinn et al. 2000; Sardelis, Turell et al. 2001).

Ticks are secondary to mosquitoes as vectors. Isolations of WN virus have been confirmed from both amblyommine (hard) and argasid (soft) ticks (Hubalek and Halouzka 1999; Campbell, Marfin et al. 2002). Compared to mosquitoes, ticks are not efficient vectors (Lawrie, Uzcátegui et al. 2004); however, Abbassy et al. (1993) proved the *Argas arboreus* tick to be competent, and according to Lawrie et al. (2004), this particular species has the ability to harbor the virus for long periods of time as a secondary reservoir. Further investigation is still needed to understand the role of ticks within the transmission cycle of WN virus (Hubalek and Halouzka 1999). See appendix A to view a full listing of known WN virus vectors.



### **2.4.3 RESERVOIR**

Although some birds do succumb to infection, neutralizing antibodies against WN virus have been found among wild and captive species (Work, Hurlbut et al. 1955), meaning they can develop immunity to infection. With a high population density, immunity against infection, and the ability to harbor WN virus for long periods of time throughout the year, birds are considered a primary reservoir. Unlike birds, lemurs do not develop clinical symptoms, but they do support the virus as a reservoir within a lemur-mosquito-lemur cycle, threatening outbreaks among mammals in forested areas (Rodhain, Petter et al. 1985). The *Rana ridibunda* species of frog has also been known to harbor WN virus and is thought to be a reservoir for *Cx. pipiens* (Hubalek and Halouzka 1999); however, it is unknown if these mosquitoes will actually feed upon these cold blooded animals [CITE Jorge Arias].

### **2.4.4 HOSTS**

WN virus has caused infection in horses, sheep, rodents, pigs, rhesus and bonnet monkeys (Hubalek and Halouzka 1999), camels, and goats (Peiris and Amerasinghe 1994); however, most mammals, including humans, are dead-end hosts. They do not circulate a high enough concentration of WN virus in their blood to infect mosquitoes and therefore cannot play a role in its transmission (Goldblum, Sterk et al. 1957).

### **2.4.5 TRANSMISSION**

The natural transmission of WN virus to a susceptible host is primarily dependant on the mosquito vector (Taylor and Hurlbut 1953); however, the primary route of transmission sustaining WN virus within the ecosystem is within a bird-mosquito-bird cycle (figure 2-3) (Work, Hurlbut et al. 1955). There are three essential processes that occur based on this cycle:

an infected mosquito bites and infects a bird; another mosquito bites the bird and in turn becomes infected; the infected mosquito bites and transmits the virus to the host. There are two cycles of bird-mosquito-bird transmission: the sylvatic, or rural, cycle involves wild birds and largely bird-feeding mosquitoes; and the synanthropic, or urban, cycle involves domestic birds and common house mosquitoes (Hubalek and Halouzka 1999). The rate of transmission

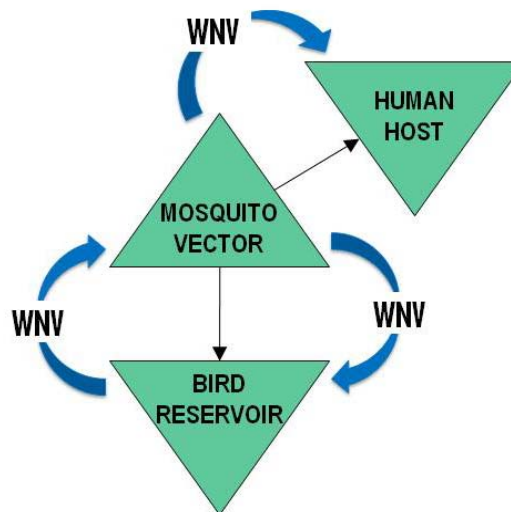


Figure 2-3: Primary WNV virus transmission cycle: bird-mosquito-bird cycle.

Adapted from: (Centers for Disease Control and Prevention 2005)

among these cycles appears to be dependent upon the population density of both mosquitoes and birds, along with changing weather patterns (Taylor, Work et al. 1956).

WN virus cannot be transmitted from person-to-person, but the virus can be spread through blood transfusions and organ transplants if the donors are not properly screened (Centers for Disease Control and Prevention 2005). Recent evidence suggests that alligators may be able to directly transmit the virus between one another. From 2001 to 2003, U.S. alligator farms showed a great economic loss due to WN infection among juvenile alligators; however, their true role in the transmission cycle is yet to be determined (Klenk, Snow et al. 2004).

#### 2.4.6 CLINICAL FEATURES & MANAGEMENT

WN virus is assumed to replicate at the position of inoculation, further spreading into the lymph nodes and the bloodstream (Diamond, Shrestha et al. 2003). Severe infections can cause destruction of neurons deep within the brain, brainstem, and spinal cord, which may cause paralysis, pathologic changes, and even death (Hayes, Sejvar et al. 2005). At this time there are

no known indicators as to whether or not a person will develop symptoms from exposure to WN virus (Centers for Disease Control and Prevention 2005). As shown in table 2-1, approximately 80 percent of human cases of WN infection may be asymptomatic and therefore go unreported; within 2-6 days from the time of exposure, the remaining 20 percent experience mild symptoms of WN fever; and within 3-14 days less than one percent will experience severe symptoms associated with WNND: meningitis, encephalitis, encephalomyelitis, and meningoencephalitis (Campbell, Marfin et al. 2002; Centers for Disease Control and Prevention 2005). According to the CDC (2005), meningitis is an inflammation of the membrane around the brain and spinal cord; encephalitis is the inflammation of the brain; encephalomyelitis is the inflammation of the brain and spinal cord; and most severe, meningoencephalitis is the inflammation of the brain, the spinal cord, and the membrane around them.

Patients diagnosed with WN fever are rarely hospitalized, but their symptoms can persist for months at a time, attacking even the healthiest of individuals (Centers for Disease Control and Prevention 2005). Infections involving the central nervous system are more critical, and the severity of the symptoms can increase depending upon the diagnosis. Symptoms associated with

Table 2-1: Symptoms associated with WN infection.

<b>INFECTION TYPE:</b>	A	F	M	E/EE/ME
<b>% OF CASES:</b>	80%	20%		<1%
<b>INCUBATION PERIOD (days):</b>	na	2-6		3-14
<b>SYMPTOMS:</b>				
Fever		x	x	x
Acute Headache		x	x	x
Body Aches		x	x	x
Muscle Weakness		x	x	x
Vomiting		x	x	x
Swollen Lymph Nodes		x	x	x
Skin Rash on Torso		x	x	x
Chills		x	x	x
Neck Stiffness			x	x
Numbness			x	x
Fatigue			x	x
Paralysis			x	x
Stupor			x	x
Disorientation			x	x
Sensitivity to Light			x	x
Permanent Damage			x	x
Tremors			x	x
Convulsions				x
Seizures				x
Coma				x
Death				x

A = Asymptomatic, F = Fever,  
M = Meningitis, E = Encephalitis,  
EE = Encephalomyelitis, ME = Meningoencephalitis

WN meningitis usually last several weeks; there have been no reported deaths among patients, but the neurological effects can cause permanent damage (Centers for Disease Control and Prevention 2005). Most deaths from WN infection in the US were reported among patients diagnosed with the most severe forms of the disease; encephalitis, encephalomyelitis (common among equine), and meningoencephalitis (Campbell, Marfin et al. 2002; Centers for Disease Control and Prevention 2005). Patients with WN meningoencephalitis are quickly hospitalized and will likely require pain medication, rehydration, breathing assistance (Hayes, Sejvar et al. 2005), and prevention of the secondary bacterial infections that have often resulted in death (Marfin and Gubler 2001). At this time, no medications have been found to fight off WN infection, and treatment for the disease will likely remain supportive (Marfin and Gubler 2001).

#### **2.4.7 PREVENTION**

Following the 1999 WN epidemic in New York, a plan called the National West Nile Virus Surveillance System was developed in the US, and it has been in place since April 1, 2000. This is a collaborative effort between the Department of Health and Human Services, the United States Geological Survey (USGS), the CDC, the United States Department of Agriculture (USDA), state and county public health departments, and natural resource agencies to monitor, prevent and control the spread of WN virus. The system develops programs that include regular testing of mosquito pools, dead birds, sentinel animals, humans, and other mammals (Dauphin, Zientara et al. 2004). These data are analyzed by the federal, state and county health departments who, in turn, implement mosquito control programs and update websites to educate the public about the disease and their part in its control (Centers for Disease Control and Prevention 2005). Experts gather at annual conferences to share surveillance and research data

in hopes of gaining a better understanding about the progression of this virus and to discuss the lessons learned during the seasonal outbreaks over the years (Tyler 2001).

Currently, there are two vaccinations being used to prevent WN infection in horses, but although scientific studies are underway, a human vaccine has yet to be developed (Hayes, Sejvar et al. 2005). All blood and organ donations are carefully monitored for WN virus, reducing the risk of infection from blood transfusions and organ transplants (Centers for Disease Control and Prevention 2005). Although there is no evidence that the virus can spread from the consumption of an infected animal, the CDC (2005) recommends following basic meat cooking rules. Preventative measures for the general population include emptying all containers holding water that could be potential breeding sites for mosquitoes and avoiding the bite of a mosquito by wearing protective clothing and using mosquito repellants with DEET.

## **2.5 EPIDEMIC HISTORY**

First isolated in 1937 near the City of Omogo, in the West Nile district of Uganda (Smithburn, Hughes et al. 1940), WN virus has reemerged and spread within the tropical and temperate regions of more than 40 countries (Campbell, Marfin et al. 2002). The approximate distribution of WN virus in 1998 is shown in figure 2-4 (Staples 2009). Broadly defined to include much of Africa, Europe, and Southeast Asia (shown in orange), seasonal outbreaks of WN infection are actually confined to a much smaller area. Based on well-documented outbreak and serological reports, the selected study areas (shown in gold) will provide a better understanding about the actual distribution of WN virus, the history of its spread, and significant findings in regards to the vector, reservoir, and host populations.

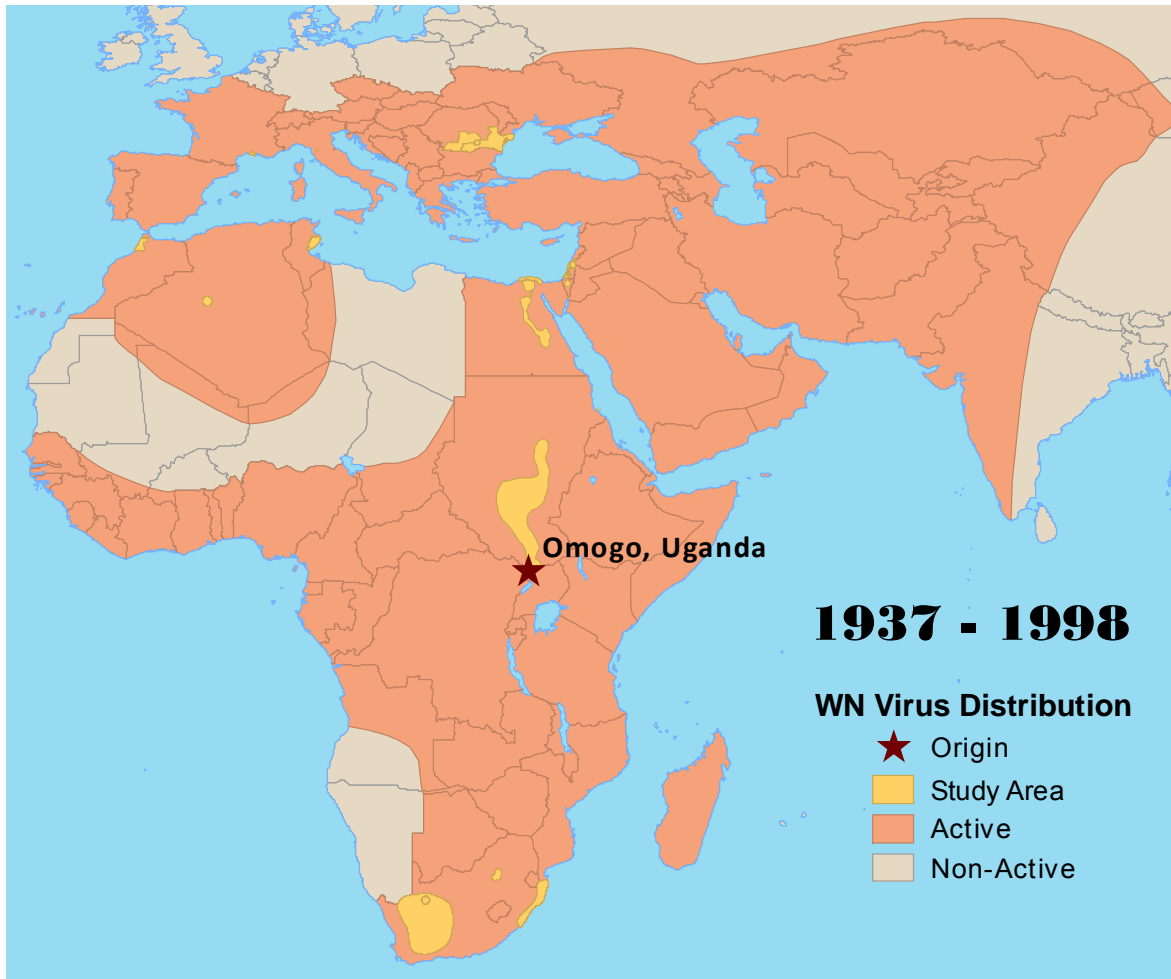


Figure 2-4: Approximate distribution of WN virus, 1937 to 1998.

Adapted from: (Staples 2009)

### EGYPT & SUDAN 1950'S

In 1950, WN virus was isolated in Egypt from the blood of three children living in villages 30 kilometers north of Cairo sparking interest in local researchers (Melnick 1951). Several studies were underway to find the competent vectors, susceptible hosts, and possible reservoirs for WN virus, and to determine the transmission dynamics of its disease. Taylor et al. (1956) tested several arthropods for vector competence, and found that only the mosquito met the requirements to sustain and transmit WN virus. Due to its abundance, high infection rate,

and the fact that it is highly ornithophilic, the *Cx. univittatus* species is the dominant vector in this region. This species feeds regularly on birds and mammals, and it is widespread throughout the Upper Nile. Also a bird and quadruped feeder, *Cx. antennatus* commonly bites humans and is thought to be the dominant vector in Southern Sudan. As a secondary vector, *Cx. pipiens* was

Table 2-2: Percentage of human blood samples testing positive for NT antibodies.

Zone	Region	Age			
		0-14	15+	All	
1	Non-endemic	Delta Perimeter	2%	39%	24%
2	Transitional	Cairo & Southern Sudan	16-28%	48-73%	40-60%
3	Endemic	Delta Center & Upper Egypt	63-70%	88-95%	77-84%

found to harbor the virus during winter months as it maintained activity within local homes.

Infection was widespread within Egypt with antibodies present among 70-80% of the human population aged 4 years and over (Melnick 1951). Increased adult immunity has made WN infection mainly a childhood disease in endemic zones. Serological studies performed by Taylor et al. (1956) from 1952 to 1954 identified different endemic zones along the Egyptian Nile and in the Southern Sudan, based on the percentage of complement-fixing (CF) and neutralizing (NT) antibodies present among the sample population (table 2-2). This is significant because CF antibodies disappear over time indicative of recent infections, whereas NT antibodies are more permanent and build up over time, suggesting infections occur regularly each year.

### **ISRAEL 1941 - 1957**

Although unconfirmed, it is believed that the first major outbreak of WN fever occurred in 1941, when 500 residents along the west coast of Israel became infected (Weinberger, Pitlik et

al. 2001). This was the first outbreak of WN fever that targeted children. Out of 303 residents of the Ma'ayan Tzvi kibbutz, a communal agricultural settlement, WN infection was confirmed among 127 children under the age of 16 Out of (Murgue, Murri et al. 2001; Weinberger, Pitlik et al. 2001).

The first outbreaks of severe WN infection were reported in Hadera Israel in 1957. Targeting the elderly, seventy-five percent of reported cases were diagnosed as clinical encephalitis resulting in four deaths (Murgue, Murri et al. 2001; Weinberger, Pitlik et al. 2001). Scientists believe the first reported deaths may be from the emergence of a new, more severe strain of WN virus. The primary vectors in this region included the *Cx. mosquito* species, mainly *Cx. pipiens* and *Cx. perexiguus*, and the reservoir birds included pigeons, storks, crows, and geese (Weinberger, Pitlik et al. 2001).

### **FRANCE 1962**

A small outbreak of West Nile virus occurred in southern France in 1962 (Panthier, Hannoun et al. 1968), affecting both horses and humans (Del Giudice, Schuffenecker et al. 2004). Before this occurrence, West Nile virus was not a common threat within Europe (Tsai, Popovici et al. 1998), and it would be many years before another epidemic would appear on the continent (Murgue, Murri et al. 2001). Most of the cases were located in the Camargue region, known for its numerous ponds, which host colonies of local and migratory birds, as well as a very large mosquito population (Dauphin, Zientara et al. 2004).

### **SOUTH AFRICA 1962-1984**

Outbreaks from WN virus can be triggered by heavy rains followed by high temperatures. Such conditions occurred in the semi-arid desert of Karoo, South Africa during the summer of 1974; heavy rains, with nearly six times higher than average precipitation received, followed by



elevated temperatures sparked the largest recorded outbreak from WN virus, with 18,000 estimated and 3,000 confirmed clinical cases of infection (McIntosh, Jupp et al. 1976). The dominant vector was the *Cx. univittatus*, which normally feeds in the canopy of the trees. Therefore, humans are not its primary food source, which explains the low number of human cases annually caused by this species (Jupp 2001). Excess precipitation increases the breeding habitat, causing the mosquito population to explode, and above average temperatures amplify viral replication within the mosquito, increasing its rate of infection. The increased population density forced the *Cx. univittatus* down from the trees into closer contact with humans, and their high infection rate probably contributing to the high number of infections among humans during these conditions (Jupp 2001).

In 1984, unusually high rainfall from October through December followed by above average temperatures from January through March sparked an epidemic/epizootic outbreak in the Witwatersrand-Pretoria region, a cool grassland located in the northeast corner of the Highveld (Jupp, Blackburn et al. 1986). Although WN infections are reported annually, the cases are more sporadic in this location than those in Karoo (Jupp, Blackburn et al. 1986). Incidence in KwaZulu-Natal, a lowland plain on the Southeast Coast, is much lower (Jupp 2001). This is probably due to the replacement of the *Cx. univittatus* (39/1000 infection rate) with the less efficient vector *Cx. neavei* (0.8/1000 infection rate) (McIntosh, Jupp et al. 1976).

### **ALGERIA 1994**

During the months of August and September, abnormal rains and temperatures triggered the emergence of severe WN infection in the semi-arid Timimoun oasis in central Sahara Algeria (Dauphin, Zientara et al. 2004). Among a total of 50 cases reported, 20 patients were diagnosed with clinical encephalitis resulting in eight deaths (Murgue, Murri et al. 2001; Dauphin, Zientara

et al. 2004). This was the first report of WN virus in this area, and with 13 patients between the age of ten months and nine years, this was the first outbreak of severe WN infection among children (Murgue, Murri et al. 2001; Dauphin, Zientara et al. 2004).

### **ROMANIA 1996**

Between the months of July and October 1996, Bucharest, Romania and the lower Danube valley of southeast Europe experienced a predominantly urban West Nile epidemic, with 61% of reported cases contracted within urban localities (Campbell, Ceianu et al. 2001). Peaking in the first week of September, this outbreak produced mainly neurological infections (Tsai, Popovici et al. 1998). Because the implementation of national surveillance was delayed and lab samples from infected patients were contaminated, the actual number of those infected may have been much higher than reported (Tsai, Popovici et al. 1998; Campbell, Ceianu et al. 2001). A total of 527 clinical cases of West Nile virus resulted in 40 deaths (LeGuenno, Bougermouh et al. 1996; Tsai, Popovici et al. 1998). The case fatality rate was 4.3% (Hayes 2001), and the incidence and fatality rates appeared to increase with age. It was documented that all those who perished were over the age of 50 (Tsai, Popovici et al. 1998).

Mosquito collection and testing led scientists to believe the most likely vector was *Cx. pipiens*, which primarily fed on migrating birds (Tsai, Popovici et al. 1998; Campbell, Ceianu et al. 2001). It is believed that poverty and poor urban infrastructure created the perfect breeding grounds for the mosquito to thrive. Many homes lacked windows or screens, facilitating contact between mosquitoes and humans (Campbell, Ceianu et al. 2001). Furthermore, a dry spell in the weather pattern was another factor that contributed to breeding habitat. When any amount of precipitation would fall, water would collect and stand in both natural and human-made containers, allowing the mosquito to reproduce (Campbell, Ceianu et al. 2001).

### **MOROCCO 1996**

From August through mid-October 1996, severe WN infection targeted equine in Morocco (Tber 1996). Approximately 50 percent out of 94 reported cases either succumbed to infection, or had to be euthanized. Breed and age did not appear to be a factor in the fatality rate, but the younger animals were able to recover faster than the adults. Most cases occurred near the Maamora forest and in heavily irrigated areas of Kenitra and Larache provinces. Renowned for arboriculture and cereal production, the region of Gharb is very humid and the climate is supportive of a large mosquito population. At the time of the outbreak, storms had produced higher than normal rainfall (Tber 1996).

### **TUNISIA 1997**

Between the months of September and December of 1997, the first WN encephalitis outbreak in Tunisia occurred in the districts of Sfax and Mahdia (Murgue, Murri et al. 2001; Feki, Marrakchi et al. 2005). A total of 173 people were hospitalized with severe encephalitis and meningitis, resulting in eight deaths (Murgue, Murri et al. 2001). Those with West Nile encephalitis tended to be much older than those with meningitis, and three of these patients, all over the age of 60, died shortly after infection (Murgue, Murri et al. 2001; Feki, Marrakchi et al. 2005).

### **ITALY 1998**

Severe cases of WN encephalomyelitis among 14 race horses occurred from August through October 1998 in Toscana Italy (Cantile, Di Guardo et al. 2000). The actual number of cases was likely underestimated due to unreporting, which was admitted from multiple horse owners (Cantile, Di Guardo et al. 2000). Most cases were reported near Padule di Fucecchio, the largest wetland in Italy, which is home to a wide variety of migratory birds (R. Romi 2004).

Climatic conditions, soil, and vegetation provides adequate habitat for large mosquito populations; dominantly *Cx. pipiens*, and *Cx. impudicus*, a partial ornithophilic species, that probably maintains the bird-mosquito-bird cycle in the spring (R. Romi 2004).

## 2.6 WEST NILE VIRUS ACTIVITY IN THE UNITED STATES

WN virus has established itself throughout the conterminous US, infecting thousands of birds, humans, and other mammals with annual seasonal outbreaks. This section will review the history of WN emergence in the Western hemisphere after the virus arrived in New York in 1999. WN virus activity has been well documented from 1999 to 2008 through the implementation of surveillance programs. The evaluation of these data highlights the actual impact of WN infection within the US over the last 10 years.

### 2.6.1 NEW YORK 1999

WN virus emerged into the Western hemisphere, with the first cases appearing in New York in the late summer of 1999 (Anderson, Andreadis et al. 1999; Lanciotti, Roehrig et al. 1999; Hayes 2001). Like the outbreak in Romania (1996), this was an urban epidemic, which spread into counties and states surrounding New York City, including New Jersey, Connecticut and Maryland by the end of 1999 (figure 2-5) (Lanciotti, Roehrig et al. 1999). Out of 719

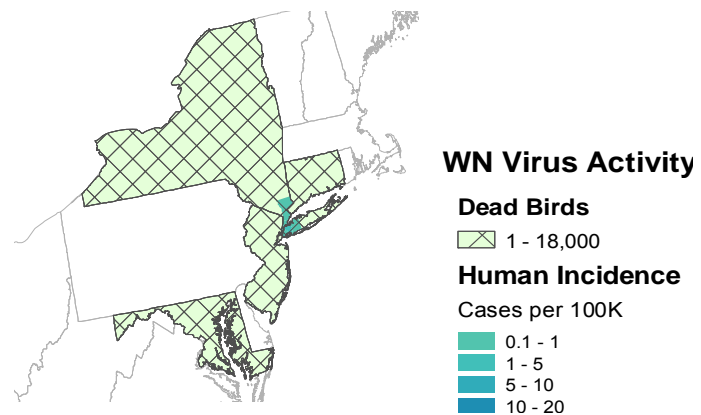


Figure 2-5: US WN virus activity in 1999.

Data Source: (Centers for Disease Control and Prevention 2000)

suspected cases of human WN infection (Jia, Briese et al. 1999; Lanciotti, Roehrig et al. 1999), 59 patients were hospitalized with severe symptoms of WN meningoencephalitis, with seven cases resulting in death (Hayes 2001). The severity of infection increased with age; the median age of those hospitalized was 71, and those over the age of 75 were at the greatest risk for death (Nash, Labowitz et al. 2001). All of the patients were relatively healthy prior to exposure, yet the case fatality rate was approximately 11% (Nash, Mostashari et al. 2001), nearly three times the case fatality rate in Romania. Reports of fatalities among other animals (Hayes 2001) include a cat, skunk, squirrel, chipmunk, rabbit, horses, and bats (United States Department of Agriculture 2001). More importantly, the New York and Connecticut state Departments of Health reported 18,379 dead birds (Eidson, Komar et al. 2001; Farnon 2006), including 12 domestic and 8 exotic captive species in the local zoos (Eidson, Komar et al. 2001).

According to Jia et al. (1999) and Hayes (2001), it is possible that WN virus had been in North America before the 1999 outbreak; however, the severity of the human cases and the high number of avian deaths associated with this epidemic is consistent with a more recent introduction. With two international airports located near the epicenter of the epidemic, the arrival of WN virus was likely through the unintentional or illegal importation of infected birds, mosquitoes (Jia, Briese et al. 1999), or domestic pets (Lanciotti, Roehrig et al. 1999), en route from endemic areas in the Mediterranean to the US (Lvov, Butenko et al. 2000).

## **2.6.2 US SURVEILLANCE, 1999 TO 2008**

From 1999 to 2008, WN virus has spread to 48 states and the District of Columbia, has been found in as many as 2,531 counties (figure 2-6). The only two states not yet affected are Hawaii and Alaska; however, it may be only a matter of time until WN virus reaches those areas as it has expanded into new territory each year including Canada, the Caribbean, Mexico, and

Central and South America (Carlson 2001). The US counties shown in gold in (figure 2-6) reported no WN virus activity over the past 10 years. This could be due to the lack of surveillance; however, these no-activity zones should be examined for possible factors and processes that limit

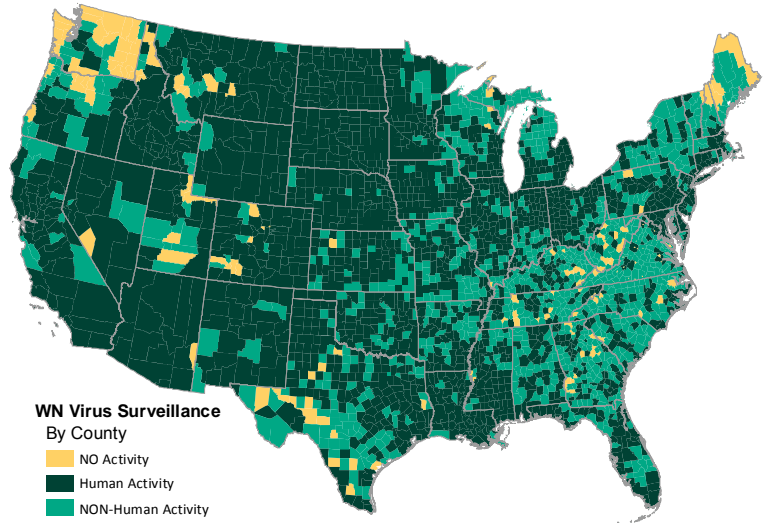


Figure 2-6: West Nile virus Activity in the US from 1999 to 2008.

Data Source: (Centers for Disease Control and Prevention 2009; United States Geological Survey 2009)

the transmission of WN virus and the spread of its disease. The US counties in dark green have reported infection among humans, and light green primarily represents infection among birds and

Table 2-3: WN Virus Activity in the US 1999 to 2008.

YEAR	*AVIAN		VET		**HUMAN						
	SPECIES	DEATHS	CASES	DEATHS	CASE TYPES			TOTAL CASES	TOTAL DEATHS	DEATH RATE	
					Fever	ND	Other			Overall	CNS
1999		18,000	25	9	3	59	0	62	7	11.3%	11.9%
2000	76	4,288	65	23	1	19	1	21	2	9.5%	10.5%
2001		7,116	733	UKN	2	63	1	66	9	13.6%	14.3%
2002	157	15,888	14,571	UKN	1,210	2,946	47	4,203	284	6.8%	9.7%
2003	225	11,330	5,145	UKN	6,996	2,866	166	10,028	264	2.6%	9.2%
2004	226	7,281	1,441	UKN	1,391	1,148	128	2,667	100	3.8%	8.7%
2005	240	5,332	1,139	UKN	1,691	1,309	99	3,099	119	3.8%	9.1%
2006		4,106	1,121	UKN	2,774	1,495	194	4,463	177	4.0%	11.8%
2007		2,182	507	UKN	2,403	1,227	63	3,693	124	3.4%	10.1%
2008	284	3,025	218	UKN	679	640	51	1,370	43	3.1%	6.7%
TOTAL	284	78,548	24,240	32+	17,150	11,772	750	29,672	1,129	5.97%	10.1%

\*AVIAN = Approximately 25% of dead birds were tested; 40-50% tested positive for WN virus.

\*\*HUMAN = Approximately 5% of actual infections were reported.

Data Sources: (Centers for Disease Control and Prevention 2009; United States Geological Survey 2009)

equine. Table 2-3 summarizes the overall impact of WN virus on populations within the US over the past 10 years, through the surveillance of collected dead birds, veterinarian reports, and diagnosed human cases.

## **HUMANS**

Using expert knowledge on mosquito-borne diseases, attempts have been made to predict where the virus would reappear each year, but it has spread much further and faster than officials had expected (Centers for Disease Control and Prevention 2006). Ten years of seasonal epidemics have resulted in nearly 12,000 cases of WN ND and 1,129 deaths (Centers for Disease Control and Prevention 2009). Adults over the age of 50 are at the greatest risk for developing WN ND, which is more likely to result in death than other WN complications (Staples 2009).

Serological surveys performed during and after seasonal outbreaks estimate that for each human case of WN ND, there are approximately 140 infections and 28 cases of WN fever (Staples 2009). In the ten year span from 1999 through 2008, estimates suggest that WN virus has infected approximately 1.65 million, causing 331,000 cases of WN fever, while confirmed reports show only 29,672 and 17,150 respectively (Staples 2009).

The geographic spread of WN virus in the US from 2000 to 2008 is shown in figures 2-7 and 2-8. According to Davis et al. (2005), WN virus likely entered the South from 2000 to 2001 traveling along bird migratory routes (figure 2-7). It then diffused westward, greatly impacting the Midwest in 2003 (figure 2-8). Compared to WN activity in the east, the presence of WN virus in the Midwest is noticeably stronger in incidence and distribution. Examining the annual trend of total cases there appears to be two waves of epidemic activity. In 2002 and 2003, the incidence of human cases and deaths significantly increased into the thousands and the virus spread into more states and counties than the previous three years combined. After a drastic

decrease in incidence in 2004, a second wave of epidemic activity peaked in 2006. With continuous activity over the past 10 years, the current trend in incidence may mean that WN virus is stabilizing as an endemic, rather than epidemic, threat in the US.

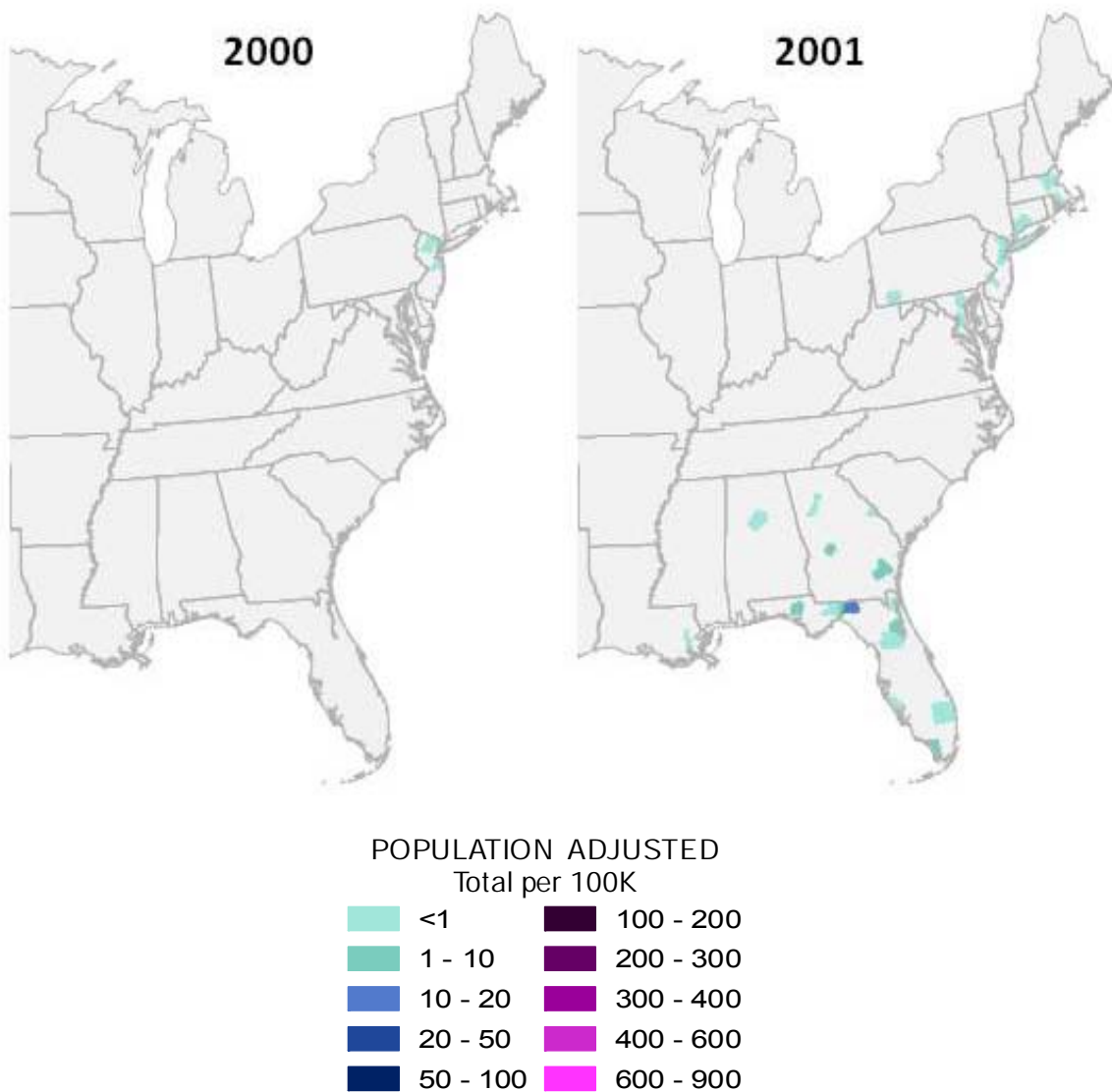


Figure 2-7: Human Cases of WN Infection in the US, in 2000 and 2001.

Data Source: (United States Geological Survey 2009)



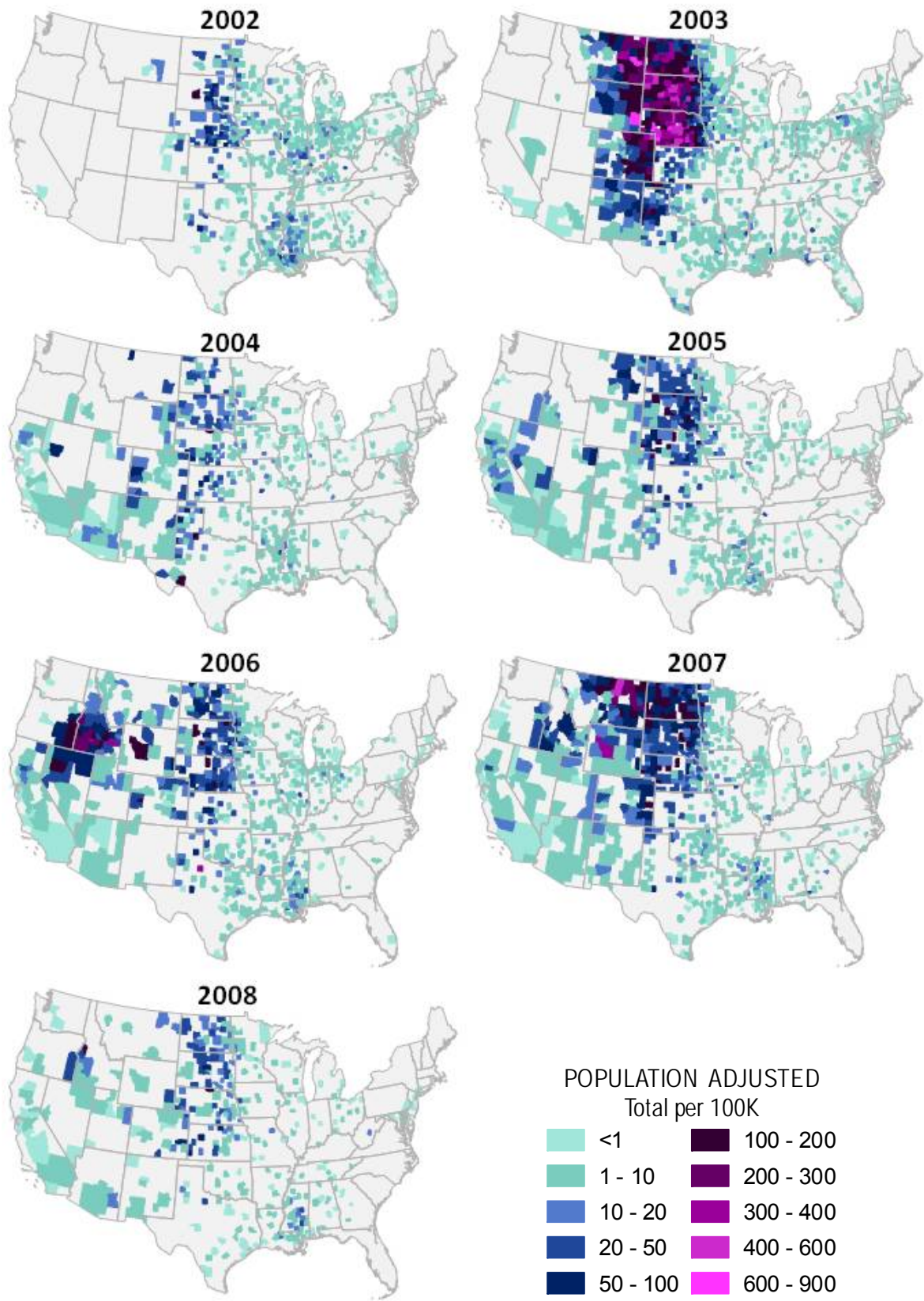


Figure 2-8: Human cases of WN infection in the US, 2002 to 2008.

Data Source: (United States Geological Survey 2009)

## DEAD BIRDS

Of the 284 avian species affected by WN virus (Peterson, Vieglais et al. 2003), 240 are native to North America. Over 80% of dead birds reported in the US were in the family Corvidae, and over 30% of them were American Crows

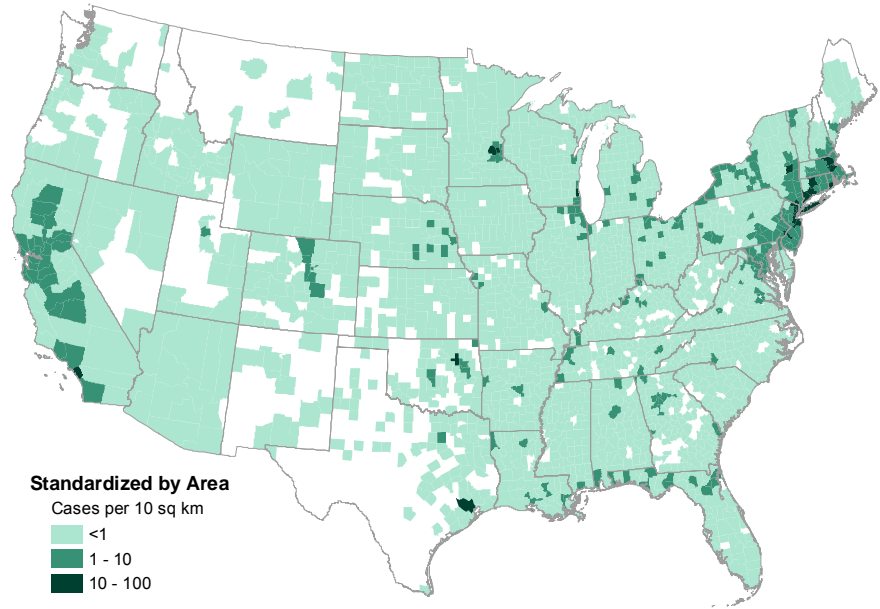


Figure 2-9: Dead Birds testing positive for WN virus.

Data Source: (United States Geological Survey 2007; Centers for Disease Control and Prevention 2009)

(Feron 2006; Centers for Disease Control and Prevention 2007). Only 26.5% of collected dead birds were tested; with nearly half of them testing positive for WN virus, the actual impact on birds is estimated at 314,000 deaths [CITE]. The location of dead birds has been associated with a 2-day lag in reports of human cases of WN infection within the same area (Campbell, Marfin et al. 2002). Figure 2-9 shows the areas where the largest numbers of dead birds were reported; based on County totals, the map represents the number of dead birds collected within a 10 square kilometer area.

The decline in the number of dead birds recorded annually from 2002 to 2007 (table 2-3) could represent a decrease in collections, or an increase in immunity to WN virus. If birds develop resistance to infection, the ability to rely on them for future WN virus surveillance programs will be eliminated (Campbell, Marfin et al. 2002). With a 50% increase in the number of dead birds from the previous year, the upward shift in 2008 could represent a new trend.

Considering WN virus is may be endemic in the US, an increase in dead birds may be an indication of an above average seasonal outbreak of WN virus, or possibly the evolution of a new viral strain.

### OTHER MAMMALS

Affecting at least 29 species of mammals in the US over the past 10 years (Eidson, Komar et al. 2001; Fernon 2006), WN virus is responsible for approximately 25,000 infections, of which 99.1% were equine (United States Geological Survey 2007). Most cases

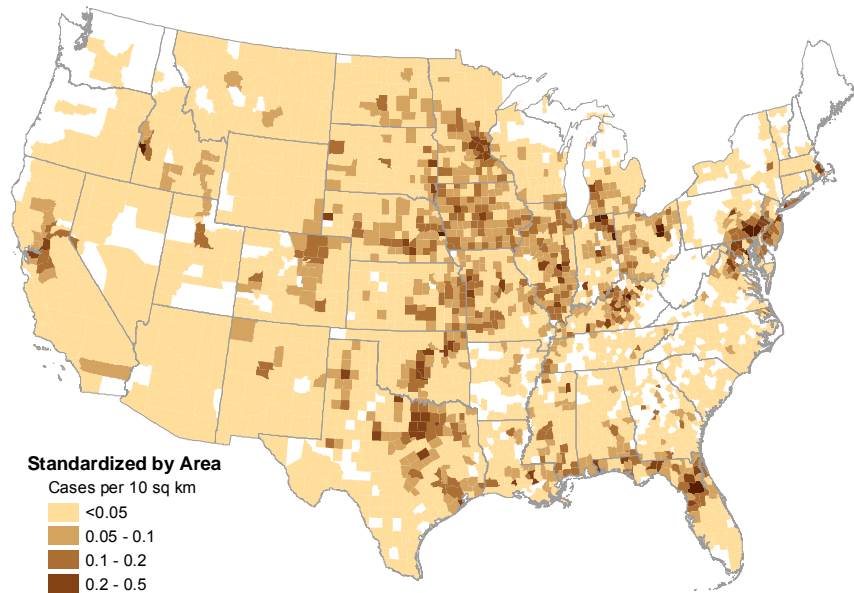


Figure 2-10: Cases of mammals testing positive for WN virus.  
Data Source: (United States Geological Survey 2007; Centers for Disease Control and Prevention 2009)

were located near in Pennsylvania, Florida, and throughout the Midwest (figure 2-10). Based on County totals, the map in figure 2-10 represents the number of other mammals, mostly equine, that were positive for WN virus within a 10 square kilometer area.

Although cases have been reported each year, the numbers have significantly declined (table 2-3), perhaps due to the availability and implementation of equine vaccinations in 2003 (Marra, Griffing et al. 2004). The condition of the US economy could play a role, especially in the upcoming season. For financial reasons, horse owners might not be able to afford vaccinations and dead animals may be disposed of without the necessary testing for disease.

## **CHAPTER 3: MANUSCRIPT**

### **A GLOBAL APPROACH TO DISEASE PREVENTION: PREDICTING HIGH RISK AREAS FOR WEST NILE INFECTION IN THE US**

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### 3.1 INTRODUCTION

As defined by Morse and Schluederberg (1990), emerging infectious diseases (EIDs) include diseases new to a population, or that have recently increased in incidence or geographic range. Nearly 75 percent of EIDs are vector-borne, meaning the transmission of the infectious agent to the host is dependent on an animal, likely an arthropod (National Center for Zoonotic 2007). Making up nearly 80 percent of known animal species and including ticks, fleas, flies, and mosquitoes, arthropods are considered the most significant vectors of disease (Gubler 2001; Black IV and Kondratieff 2005). Vector competence measures the ability of the arthropod to become infected and successfully transmit the pathogen to a susceptible host (Morse and Schluederberg 1990; Ewald 1996). The most competent arthropod vector is the mosquito; very abundant and widely distributed, mosquitoes are capable of transmitting some of the most infectious pathogens known to humans (Ewald 1996).

Eradication programs focused on eliminating the mosquito vectors ended in the 1970s, shifting health care policies of vector-borne infectious diseases from prevention to emergency response (Gubler 1998). For over 30 years the lack of preventative measures has contributed to habitat expansion; thus the distribution and density of important vector species has increased throughout the world. Globalization, shifting weather patterns, pathogenic evolution, and drug and insecticide resistance have further eliminated barriers and encouraged the spread of infectious diseases (Ozer 2005). An increased vector population, the absence of barriers, and human activities have contributed to the global emergence and reemergence of mosquito-borne diseases, which are currently responsible for over 500 million cases and 1.5 to 3 million human deaths each year (World Health Organization 2002). Some of the most important mosquito-borne EIDs of the 21<sup>st</sup> century include malaria, dengue hemorrhagic fever, and West Nile (WN) virus.

WN virus was first discovered in 1937 when it was isolated from the blood of a woman living in the West Nile district of Uganda (Smithburn, Hughes et al. 1940). For over 60 years the virus spread, creating endemic and epidemic regions throughout Africa, Europe, and Southwest Asia (Hayes 2001; Dauphin, Zientara et al. 2004). Crossing the Atlantic Ocean in 1999, WN virus emerged in New York City making its first debut in the Western hemisphere (Campbell, Marfin et al. 2002). Invading territories previously free of its disease, this exotic pathogen spread faster and further than public health officials expected (Carlson 2001), making a significant impact on human, equine, and avian populations throughout the US and surrounding territories (Staples 2009).

With ten years of consistent seasonal activity, WN virus has spread and established itself throughout the conterminous US, and it has become the number one cause of neuroinvasive disease among humans (Staples 2009). Approximately 11,772 cases of severe WN neuroinvasive disease (WNND) and 1,129 deaths have been reported thus far (United States Geological Survey 2007; Centers for Disease Control and Prevention 2009), and according to the Centers for Disease Control and Prevention (CDC), estimates suggest 1.65 million people have likely been infected (Staples 2009).

Given that US health care policies were focused on emergency response rather than prevention and the fact that WN virus had not been a threat in the past, it is understandable that public health officials were not prepared for its emergence in 1999. However, a review of temporal and spatial distribution of human cases, incidence, and deaths from WNND suggests the US approach to the prevention of EIDs could be improved. Cases and deaths from human WNND occurred every year from 1999 through 2008 in the US (figure 3-1). The first epidemic wave, or significant increase in human cases, was clearly during the 2002 and 2003 seasons. Approximately 6,000 cases and 600 deaths from human WNND were reported during those

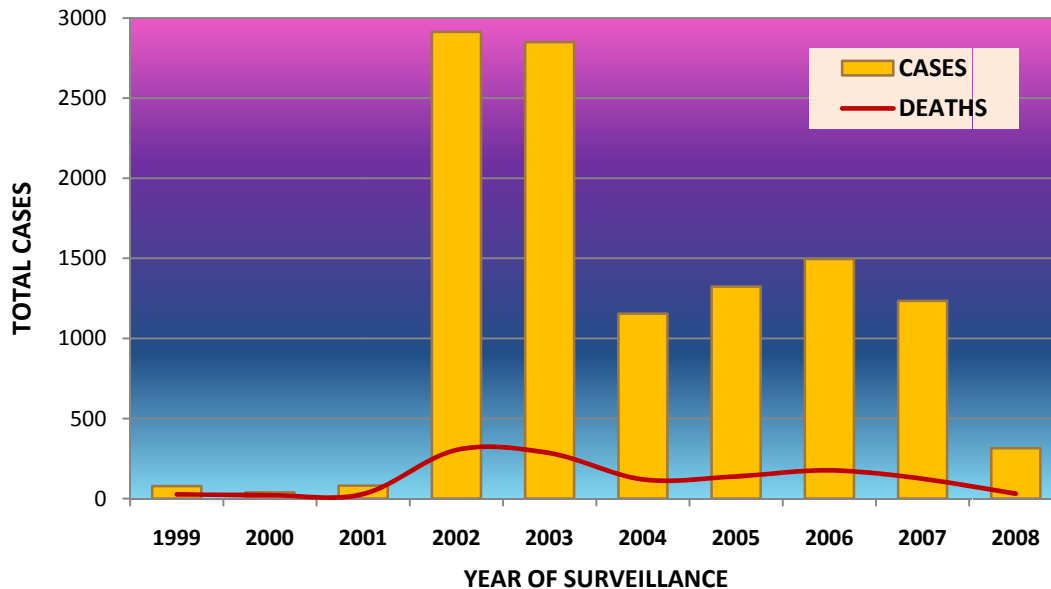


Figure 3-1: Reported human cases and deaths from WNND in the US from 1999 to 2008.

Data Sources: (United States Geological Survey 2007; Centers for Disease Control and Prevention 2009)

years. The number of cases dropped in 2004; however, it appears that another epidemic wave began during that year and continued through 2007. In total, 5200 cases and 560 deaths were reported between 2004 and 2007, and the reports from each year were similar in numbers; yearly case totals were between 1,200 and 1,500 and deaths totals were between 120 and 177.

Given the impact EIDs can have on humans, the increased distribution and abundance of important mosquito vectors, the potential for rapid global transmission, and the threat for bioterrorism in the 21<sup>st</sup> century (Fine and Layton 2001; Layton 2001), it is essential to attain the knowledge and ability to predict the distribution of mosquito-borne infectious diseases so that public health officials can be more prepared for a rapid emergency response. It was 3 to 4 years into its spread across the US when the greatest impact of the WNND occurred. With a better understanding about WN virus, the dynamics of its transmission, and the epidemiological factors that influence the risk of human infection, could US health officials have been more prepared to control the spread and reduce the incidence of WNND among humans?

The intention of this study is to introduce a global approach to the prediction of emerging vector-borne diseases that successfully predicts high-risk areas for human infection in the US based on factors associated with WN virus activity in the Eastern hemisphere. Based on theoretical concepts from disease ecology, landscape epidemiology, and spatial epidemiology, there are two basic purposes of this study:

- (1) To complete a fine-scale analysis of environments where WN virus activity has been reported in the Eastern hemisphere (study areas) to identify the important factors and processes that define and influence the distribution of human risk for WN infection.
- (2) To develop a static model of risk based on the range of variability of each factor among the study areas to predict US counties that are high-risk areas for increased transmission of WN virus and incidence of human WNND.

The applied purpose of this study is:

- (3) To standardize a method that can be applied to predicting the risk of other vector-borne diseases in preparation to improve the rate and efficiency of their prevention and control.

Risk prediction of vector-borne diseases is very complex, involving relationships among several entities: the virus, vectors, reservoirs, and hosts (Work, Hurlbut et al. 1955). Dynamic models of risk for WN virus have focused on the monitoring of dead birds (Mostashari, Kulldorff et al. 2003; Theophilides, Ahearn et al. 2003; Roberts and Foppa 2006), or temperature degree days as early indicators of increasing risk (Ward 2005; Zou, Miller et al. 2007). Static models of risk have incorporated techniques to model the spatial distribution of mosquito habitat and the human population (Brownstein, Rosen et al. 2002; Wallis 2005; Ward, Ramsay et al. 2005; Tachiiri, Klinkenberg et al. 2006; Zou, Miller et al. 2006), municipal-level factors and human incidence (Yiannakoulis and Svenson 2007), and associations between incidence and



environmental factors. Although important to the identification and validation of significant factors, the results of these studies are area specific, meaning each is one sample of where WN activity can occur. Given that topography, land use, and climate vary throughout the world, these small-scale studies are not representative of the true epidemiological landscape of WN virus. A broader perspective is needed to predict high-risk areas for EIDs at the national level and provide public health officials with locations to direct funding for research and education in preparation for its prevention or control.

The objective the model of risk in this study is to identify US counties that are high-risk areas for incidence of human WNND based on a crisp set of landscape characteristics that are related to known areas of WN virus activity in the Eastern hemisphere. The purpose of this model is to provide a decision making tool for public health officials who are in charge of allocating funding for the prevention and control of infectious vector-borne diseases. By identifying the high-risk counties within the US, research can focus on a finer-scale analysis in preparation for efforts of prevention and control.

## **3.2 APPROACH**

Like most vector-borne diseases, the transmission of WN virus is complex, The primary transmission cycle sustaining WN virus within the ecosystem is the bird-mosquito cycle (Work, Hurlbut et al. 1955). Simply, the infected mosquito bites a bird and the bird becomes infected; birds can harbor the virus for a long period of time, infecting other mosquitoes that feed upon it. Birds play an important role in sustaining WN virus within the ecosystem (Work, Hurlbut et al. 1955); however, it is ultimately the mosquito that transmits the virus to the human host. Theoretical approaches from disease ecology, landscape epidemiology, and spatial epidemiology were applied to identify the relationships between the mosquito vector and human host.

### 3.2.1 DISEASE ECOLOGY

Disease ecology supports the study of the relationships between different populations under changing environmental conditions (Meade and Earickson 2000). As shown in figure 3-2, factors that can increase risk for human infection are based on the population, behavior, and habitat (PBH) characteristics of both the mosquito vector and the human host (Meade and Earickson 2000).

Considering mosquitoes and humans as separate entities interacting in nature, the ecological risk of WN infection is based on the varying boundaries of their habitats. The highest risk for transmission is when and where these boundaries overlap (Meade and Earickson 2000).

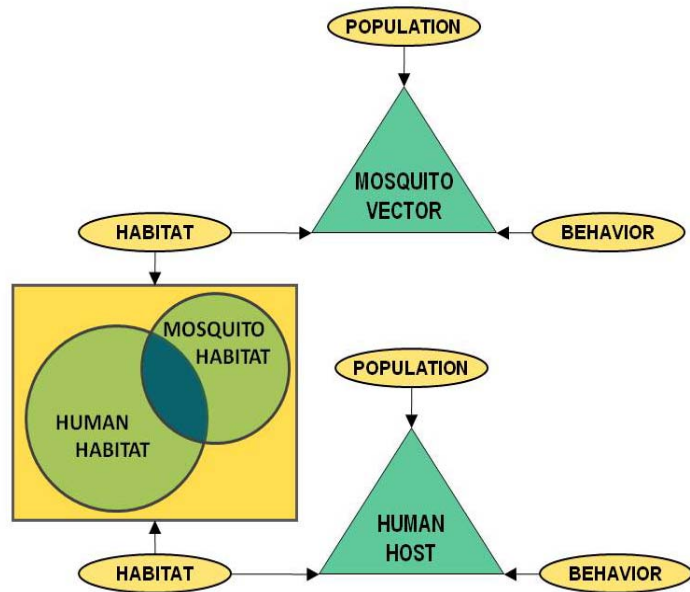


Figure 3-2: Ecological Risk of West Nile Infection.  
Adapted from: (Meade and Earickson 2000)

### 3.2.2 LANDSCAPE EPIDEMIOLOGY

Landscape epidemiology focuses on the identification of important factors that influence the existence of disease and the pathways of transmission. The goal is to discover points of intervention, where landscape modifications can be used to control the spread and incidence of disease. Theoretically, landscape epidemiology highlights the importance of gaining a multi-scale perspective to understand the dynamics of disease transmission (Meade and Earickson 2000). The natural nidus represents the ecosystem where the disease agent is sustained (Ostfeld, Glass et al. 2005), and evaluating the interactions between the natural nidus and the broader

biome is important for identifying outside influences that may affect the transmission of WN virus.

### **3.2.3 SPATIAL EPIDEMIOLOGY**

Spatial epidemiology defines the landscape epidemiology of a disease, typically using a Geographic Information System (GIS). A GIS provides the software and tools to collect, analyze, and review spatial data associated with vector-borne diseases (Moore and Freier 2005). This technology has given medical geographers the opportunity to explore the relationships between the incidence of disease; the distribution of the pathogen, vectors, and hosts; and changing environmental conditions (Meade and Earickson 2000; Moore and Freier 2005; Ostfeld, Glass et al. 2005). The dynamics of these relationships can identify the important factors and processes that can increase the risk of human infection, and offer a better understanding about prevention and control (Moore and Freier 2005).

### **3.3 THE EPIDEMIOLOGY OF WN VIRUS**

The epidemiology of a pathogen is defined by the factors and processes that influence the distribution, transmission, and risk of its disease. Factors are the static or pre-existing conditions of the PBH characteristics of the vector and host. Processes are the dynamic events or activities within the environment that influence change or variability among factors, and increase the risk of human infection. Documentation of WN virus activity in the Eastern hemisphere describes the distribution of vector species, the impact of WN infection on humans, and environmental conditions that are associated with increased transmission. This information contributed to the identification of the important factors and processes that influence the risk for human WN infection.

The epidemiology of WN virus is shown in figure 3-3. The important factors associated with the transmission of WN virus include: mosquito species; human age, gender, and susceptibility; and the habitat defined by the characteristics of climate, topography, land use, land cover, tree cover, vegetation, sunlight, soil, water, human population density, and the percent of urbanization. The known conditions of these factors in the study areas will be used to define the range of factor variables and their associated level of risk. While environmental variability, including heavy precipitation and above average temperatures, influences mosquito distributions and the probability of human exposure, ultimately it is human behaviors, such as research, education, personal protection, habitat management, and surveillance efforts that will prevent or minimize WN infection in humans.

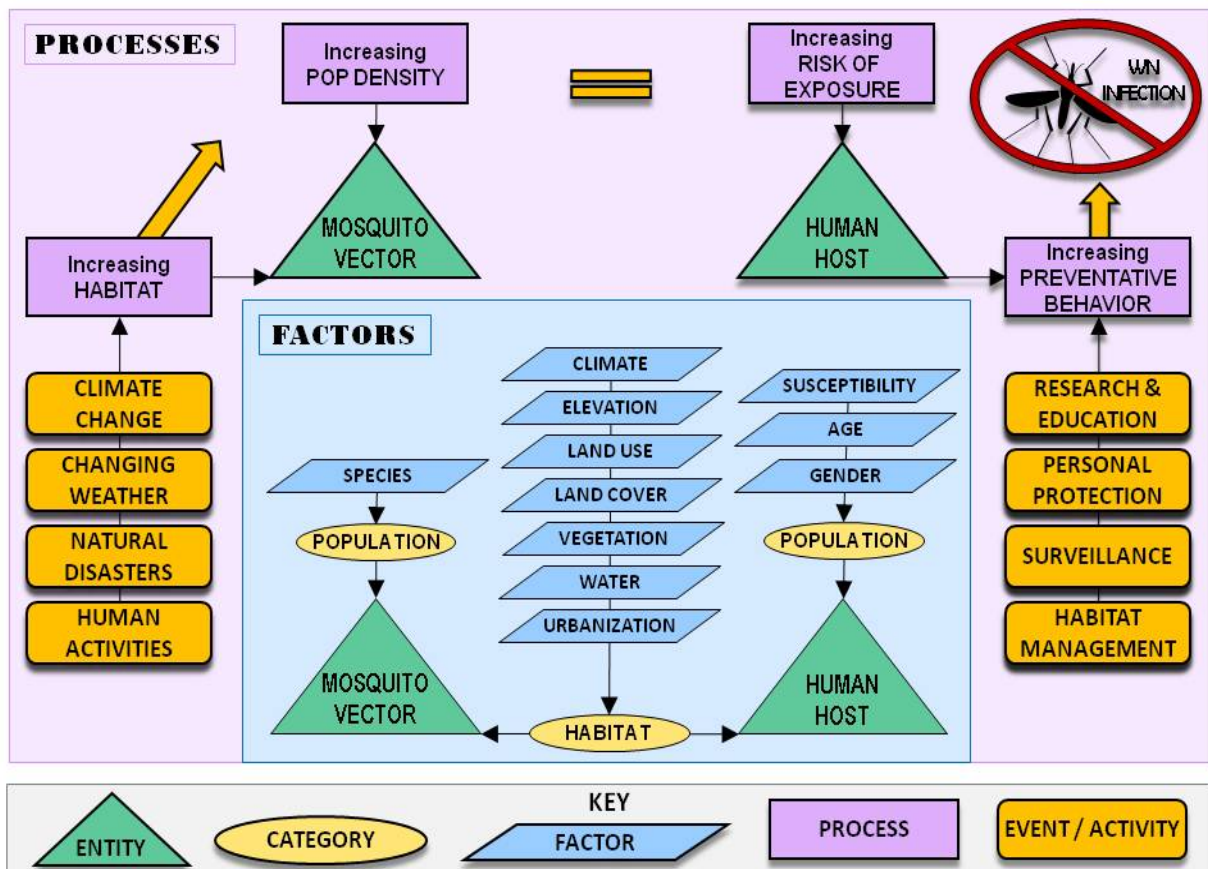


Figure 3-3: Epidemiology of WN virus.

### 3.3.1 FACTORS

#### MOSQUITO POPULATION

Mosquito behaviors, such as when and where they feed and their choice of host, are species-specific and directly related to the amount of risk for human infection (Hayes 2001). The dominant vectors of WN virus are largely ornithophilic, or bird-feeding mosquitoes (Hubalek and Halouzka 1999). Studies on vector competence measures how well the mosquito is able to become infected and successfully transmit the infectious agent to the host (Taylor, Work et al. 1956; Jupp 1974; Hubalek and Halouzka 1999; Turell, O'Guinn et al. 2000; Sardelis, Turell et al. 2001). A vector is competent if it is capable of becoming infected; however an efficient vector is able to maintain a high rate of transmission to human hosts (Jupp 2001). Important vectors for WN virus in the US include: *Culex. tarsalis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. salinarius*, *Aedes albopictus*, and *Ae. vexans* (Staples 2009).

*Cx. tarsalis*, *Cx. pipiens*, *Cx. restuans*, and *Cx. quinquefasciatus* are the most abundant vectors in US (table 3-1). The association of these species with incidence of WN infection is regional-specific. The highly efficient *Cx. tarsalis* dominates the regions west of the Mississippi (Turell, O'Guinn et al. 2002; Hayes, Komar et al. 2005; Eisen, Bolling et al. 2008); *Cx. pipiens*, and *Cx. restuans* are moderately efficient vectors are common to the Northeast (Hayes, Komar et al. 2005; Kilpatrick, Kramer et al. 2005); and the least efficient *Cx. quinquefasciatus* can be found across the Southern US (Sardelis, Turell et al. 2001; Hayes, Komar et al. 2005); better known as the common house mosquitoes of the North and South, these species tend to be more abundant in urbanized areas (Hayes, Komar et al. 2005). Breeding habitat for these species include marshes, swamps, pools, tree holes, tires, human containers, and most locations where water can collect (Crans 2004).

Table 3-1: Dominant vectors for WN virus in the US.

<b>SPECIES</b>	<b>EFFICIENCY</b>	<b>HABITAT</b>	<b>DISTRIBUTION</b>
<i>Cx. tarsalis</i>	HIGHLY	Wetland	West of the Mississippi
<i>Cx. pipiens &amp; restuans</i>	MODERATE	Container Heavily polluted	Northeast
<i>Cx. quinquefasciatus</i>	MODERATE-LOW	Container Ground pools	South

## **HUMAN POPULATION**

The incidence and severity of human infections from WN virus have been associated with age. In endemic regions, adults have typically developed immunity from years of exposure to the pathogen, and WN infection therefore tends to target children under the age of four (Melnick 1951; Bernkopf, Levine et al. 1953; Taylor, Work et al. 1956; LeGuenno, Bougermouh et al. 1996). In contrast, the population in epidemic areas has had little to no exposure to the pathogen. Without immunity, all ages are at risk for infection, and the elderly are at the greatest risk for WN encephalitis and death (LeGuenno, Bougermouh et al. 1996; Tsai, Popovici et al. 1998; Feki, Marrakchi et al. 2005). During the 1996 epidemic outbreak in Romania, the incidence and fatality rates appeared to increase with age; those who perished were over the age of 50 (Tsai, Popovici et al. 1998). During the 1997 epidemic in Tunisia, patients diagnosed with WN encephalitis, a more severe form of WNND that can result in death, tended to be much older than those with meningitis; three patients over the age of 60 died shortly after infection (Murgue, Murri et al. 2001; Feki, Marrakchi et al. 2005). It is no surprise, therefore, that during the 1999 epidemic in New York, the median age of patients diagnosed with the most severe cases of WN meningoencephalitis was 71, and those over the age of 75 were at the greatest risk for death (Nash, Labowitz et al. 2001).

WN infection tends to appear more frequently in males (Davis, Hayes et al. 2005; Staples 2009); however, incidence could be a factor of time spent outdoors. More research is needed to

determine the relationship between gender and vector-borne disease. Genetics, immunity, and the pre-existence of disease are other factors that can determine if a human will develop WN infection and may influence the severity of symptoms they will endure (Centers for Disease Control and Prevention 2005). Patients that are hospitalized with WNND are also treated to prevent secondary bacterial infections that have commonly been the cause of death (Marfin and Gubler 2001).

### **CLIMATE**

Climate is one of the main factors that defines the distribution of disease vectors (Epstein, Diaz et al. 1998; Gratz 1999). Higher temperatures support a growing population and the increased infection rate of important vector species; their overall distribution may be expanding due to global warming (Haggett 1994). Outbreaks of WN virus have occurred in semi-arid (McIntosh, Jupp et al. 1976; Jupp, Blackburn et al. 1986; Katz, Rannon et al. 1989; LeGuanno, Bougermouh et al. 1996), sub-tropical (Taylor, Work et al. 1956), and temperate climates (Panthier, Hannoun et al. 1968; Tber 1996; Tsai, Popovici et al. 1998; Cantile, Di Guardo et al. 2000; Triki, Murri et al. 2001). The relevant measurements of climate include temperature, precipitation, humidity (Khasnis and Nettleman 2005).

### **ELEVATION**

Mosquitoes exist at a range of elevations from below sea-level to 3,000 meters or more (Eldridge 2005). Mountain ranges no longer serve as barriers for mosquito-borne diseases, which are now being reported in the highlands of Asia, Africa, and Latin America (Epstein, Diaz et al. 1998). Eisen et al. (2008) sampled mosquitoes along different elevation gradients (1,500-2,400 meters) in Northern Colorado.

Important vectors of WN virus, *Cx. tarsalis* and *Cx. pipiens*, are well established in the plains (<1,600 meters; mean June-August temperature >19.5°C) and the foothills/low-montane areas (1,610-1,730 meters; 18-19.5°C); *Cx. tarsalis* is sporadic in the mid-range montane (>1,750 meters; <17.5°C) and high montane areas (2,360-2,540 meters) (Eisen, Bolling et al. 2008). Highly dependent on temperature, the geographic distribution of mosquitoes at higher latitudes and elevations increases during the warm summer months (Eisen, Bolling et al. 2008).

### **LAND USE / LAND COVER**

Pressure to feed the growing human population has increased the colonization of agricultural settlements throughout the world (Haggett 1994). Several studies in the Eastern hemisphere have found incidence of WN infection increases in agricultural areas (Melnick 1951; Bernkopf, Levine et al. 1953; Taylor, Work et al. 1956; Haggett 1994). These environments are very humid and capable of supporting a large population density of mosquitoes and birds (Taylor, Work et al. 1956). WN virus activity in Morocco was reported in the region of Gharb; renowned for cereal production, this area is heavily cultivated and irrigated (Tber 1996). Additionally, most cases in were reported near the edge of the Maâmora forest (Tber 1996), which is known to be supportive of birds and bird-feeding mosquitoes (Haggett 1994). Protected areas, usually associated with water or wetland marshes (Panthier, Hannoun et al. 1968; Cantile, Di Guardo et al. 2000), fish farming, and open ponds are attractive to thousands of migratory birds, and have been linked with increasing WN virus activity (Bin, Grossman et al. 2001).

### **VEGETATION**

The distribution of vector mosquitoes can be measured by the degree of vegetation (Brownstein, Rosen et al. 2002). *Cx. tarsalis* is found in densely vegetative areas near the edge of ponds (Zou, Miller et al. 2006), and soils can also be used to represent the boundaries of



vegetation types that attract and support the important vector species (Tber 1996). Monitored at the local level, changes in vegetation can be used to understand the distribution and population density of mosquitoes for habitat maintenance and control (Britch, Linthicum et al. 2008), and remote sensing has been used to examine the relationship between vegetation change and WN infection (Brownstein, Rosen et al. 2002; Wallis 2005; Ward, Ramsay et al. 2005).

## **WATER**

WN virus activity has been reported along the edges of river systems and in deltas that support large populations of birds and mosquitoes (Taylor, Work et al. 1956; Panthier, Hannoun et al. 1968; Tsai, Popovici et al. 1998; Savage, Ceianu et al. 1999). Zou et al. (2006) found that large bodies of water (>4ha) producing waves from exposure wind and streams with running water are not suitable mosquito breeding habitat (Zou, Miller et al. 2006). *Cx. tarsalis* and *Cx. restuans* prefer the calmness of swamp water (Shaman, Stieglitz et al. 2002), wetland marshes (Cantile, Di Guardo et al. 2000; R. Romi 2004), and the edge of ponds, including livestock watering ponds, or discharge water ponds used in coal bed methane development (Zou, Miller et al. 2006).

## **URBANIZATION**

The degree of urbanization can be measured by the density of the human population. Crowded conditions are often accompanied by an increase in pollution and a deteriorating infrastructure, which is supportive of urban mosquito habitat (Eisen, Bolling et al. 2008). The dominant species associated with the 1996 outbreak in Romania was *Cx. pipiens*; this urban species is a container breeder, known to breed where ever water can collect (Savage, Ceianu et al. 1999; Crans 2004). Suitable container habitat including tree holes, old tires (Joy and Clay 2002; Yee 2008), gutters, and potted plants (Joy and Clay 2002). As the population density of

large cities continues to rise, the lack of preventative efforts will continue to support an increasing population of urban vector species (Haggett 1994).

### **3.3.2 PROCESSES**

Environmental processes that may have contributed to epidemic outbreaks in the Eastern hemisphere include heavy rains and flooding, above average temperatures, and the natural- and human-induced expansion of mosquito breeding habitat (Hubalek and Halouzka 1999). Because these processes are dynamic, they must be evaluated at the local level to detect and respond quickly with prevention and control.

#### **CHANGING CLIMATE**

Global climate change will influence the transmission dynamics of vector-borne diseases (Zell 2004). Global warming will increase the distribution of important mosquito vectors to high latitudes and elevations (Haggett 1994; Epstein, Diaz et al. 1998; Gratz 1999; Zell 2004; Khasnis and Nettleman 2005), increasing human risk of infection (Khasnis and Nettleman 2005).

According to Epstein et al. (1998), the transmission season for mosquito-borne diseases may also be extended. The effects of ocean warming and El Nino can lead to increased precipitation, sparking epidemics where pathogenic activity does not usually exist (Epstein, Diaz et al. 1998; Engelthaler, Mosley et al. 1999; Anyamba, Linthicum et al. 2001).

#### **CHANGING WEATHER PATTERNS**

Mosquitoes are exceptionally sensitive to changing weather patterns (Epstein, Diaz et al. 1998). Excessive precipitation, above average temperature, drought, and humidity have been associated with increased WN virus activity (McIntosh, Jupp et al. 1976; Jupp, Blackburn et al. 1986; LeGuanno, Bougermouh et al. 1996; Hubalek and Halouzka 1999; Jupp 2001; Paz 2006). In 1974, an epidemic was triggered in Karoo, South Africa by above average rainfall followed by

increased temperatures (McIntosh, Jupp et al. 1976). The increased precipitation provides excellent breeding habitat for vector populations to grow, and higher temperatures increase viral replication within the mosquito; the mosquito becomes a more efficient vector of disease, able to become infected quickly and transmit a large amount of the virus to the host (McIntosh, Jupp et al. 1976; Jupp, Blackburn et al. 1986; Jupp 2001).

### **NATURAL DISASTERS**

Hurricanes, tornadoes, mass land movement, and floods are all natural disasters that can create an increase in vector breeding habitat (Hubalek and Halouzka 1999). Like swamp water, flood water can provide new habitat for mosquitoes to breed (Shaman, Stieglitz et al. 2002). Massive landscape modifications and housing destruction can provide increased habitat and increased human exposure and risk.

### **HUMAN ACTIVITIES**

Human-induced landscape modifications are conducive to the increase of mosquito habitat. Deforestation can increase the amount of forest edge habitat to support a larger population of mosquito vectors (Haggett 1994). Dam construction can provide a large stable body of water conducive to mosquito habitat (Haggett 1994). The deterioration of our infrastructure, increasing urbanization, decreasing sanitation all contribute to increased habitat for important vector mosquitoes. The *Cx. pipiens* is a container breeder that prefers polluted and highly organic water (Crans 2004).

## **3.4 METHODS**

The development and validation process used to create the predictive model of risk of human WN infection in the US is outline in this section. This information provides an overview of the selected study areas, spatial data, and the GIS processing that was involved.

### **3.4.1 STUDY AREAS**

The study areas consist of the countries where epidemic and endemic WN virus activity was reported prior to 1999. Incident reports and serological studies testing for antibodies against WN virus in human, equine and avian populations have identified multiple ecosystems in which WN virus has been able to sustain itself among eleven countries (figure 3-4): Uganda (Smithburn, Hughes et al. 1940); Israel (Leffkowitz 1942; Bernkopf, Levine et al. 1953; Goldblum, Sterk et al. 1954; Radt 1955; Marberg, Goldblum et al. 1956; Goldblum, Sterk et al. 1957; Spigland, Jasinska-Klingberg et al. 1958; Katz, Rannon et al. 1989; Bin, Grossman et al. 2001; Weinberger, Pitlik et al. 2001), Egypt (Melnick 1951; Taylor, Work et al. 1956; Schmidt and El Mansoury 1963), Sudan (Taylor, Work et al. 1956), France (Panthier, Hannoun et al. 1968), South Africa (McIntosh, Jupp et al. 1976; Jupp, Blackburn et al. 1986; Jupp 2001), Algeria (LeGuenno, Bougermouh et al. 1996), Morocco (Tber 1996), Romania (Tsai, Popovici et al. 1998; Savage, Ceianu et al. 1999; Campbell, Ceianu et al. 2001), Tunisia (Triki, Murri et al. 2001; Feki, Marrakchi et al. 2005), and Italy (Cantile, Di Guardo et al. 2000; R. Romi 2004).

Within the study areas, twenty-five study zones were isolated according to the literature based on the level of WN virus activity that has occurred. The study zones are distributed throughout semi-arid, subtropical, and temperate regions in the Eastern hemisphere, and they capture a wide variety of ecosystems. The boundaries for each study zone were created using global administrative boundaries (GADM) (Hijmans, Garcia et al. 2007), or they were digitized

based on maps and images obtained from the literature. Using a GIS, environmental data extracted from each study zone are used to identify the important factor variables explained below.

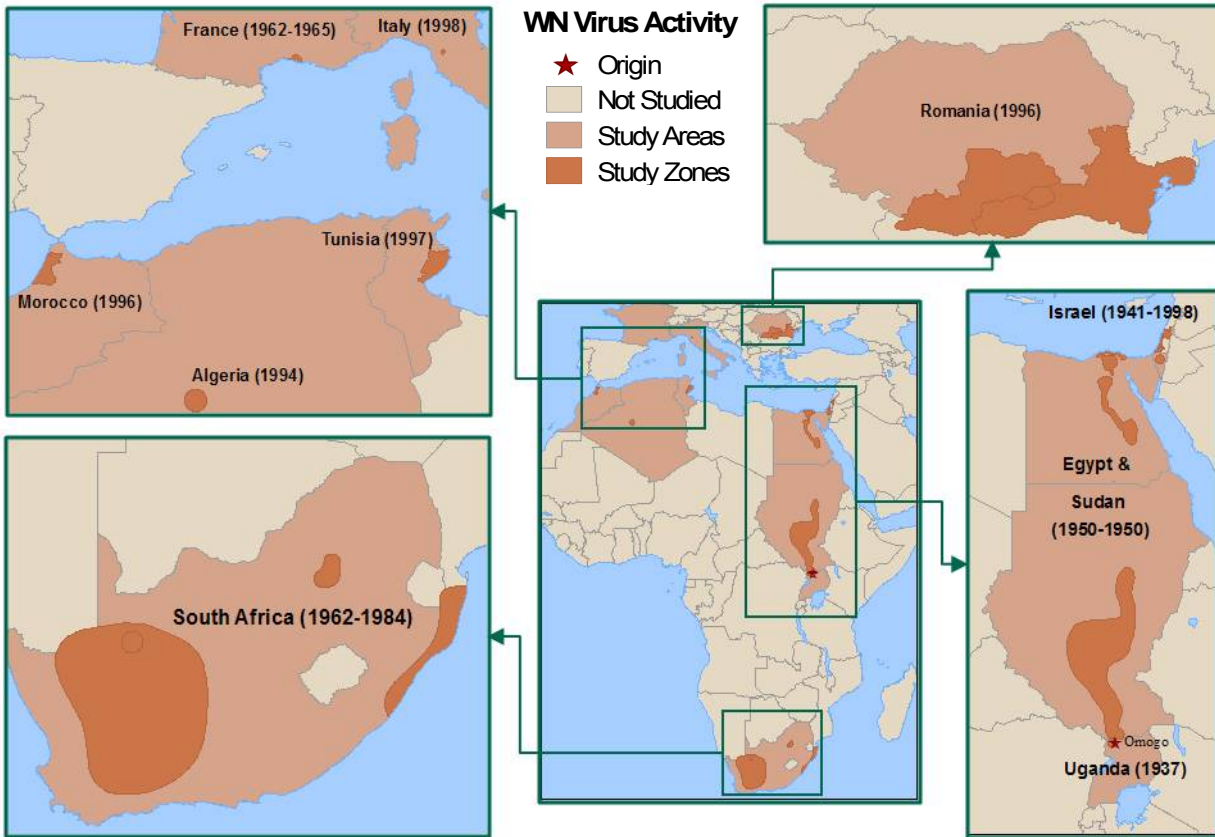


Figure 3-4: Study areas and zones in the Eastern hemisphere.

### 3.4.2 SPATIAL DATA

Spatial datasets used in this study were selected based on the quality of the data, availability at the global extent, and a minimum resolution of one kilometer (table 3-2). The datasets that met these requirements capture four of the seven habitat factors: elevation, land cover, vegetation, and climate. Mosquito species, human population, land use, water, and urbanization were not considered in this model; their elimination was based on the inability to obtain quality spatial data that met the previously described requirements.

Table 3-2: List of digital data sources.

FACTOR	DATA SOURCE	RESOLUTION
Elevation	Shuttle Radar Topography Mission (SRTM 2009)	90 m
Land Cover	Global Land Cover Classification (GLC 1998)	1 km
Vegetation	Global Tree Cover Continuous Fields (GTC 2000)	1 km
Climate	WorldClim – Weather Station Interpolated (WORLDCLIM 2004)	1 km

Elevation: Shuttle Radar Topography Mission (SRTM) elevation data is the most complete high-resolution (90 meter) elevation data available at the global scale (National Geospatial-Intelligence Agency and National Aeronautics and Space Administration 2000).

Land Cover: Global Land Cover Classification (GLCC) at a resolution of 1 kilometer, was created by the supervised classification of Advanced Very High Resolution Radiometer (AVHRR) satellite imagery (Hansen, DeFries et al. 1998). This dataset defines thirteen land cover classes including forests, woodlands, grasslands, shrublands, barren land, cropland, and urban areas.

Vegetation: Global Tree Cover Continuous Fields represents the density of vegetation (DeFries, Hansen et al. 2000). This dataset is based on vegetation of all land cover classes described by the GLCC. This data will highlight the forest edges, important to mosquito habitat.

Climate: WorldClim climate grids were created from the interpolation of global weather station data, based on 30-year monthly averages (1960-1990), with SRTM elevation (Hijmans, Cameron et al. 2005). The variables in this set include four monthly sets: total precipitation, minimum, maximum, and mean temperature. From these monthly datasets, 19 bioclimatic variables were derived, eight of which are used in this study and will be described in the GIS processing section below (BIO 5, 8, 12, 13, 14, 16, 17, 18).

### **3.4.3 GIS PROCESSING**

Given that this model represents a starting point for the control and prevention of EIDs in the US at the national scale, over prediction of risk would be acceptable, and to avoid excluding areas of risk, barriers were not considered. The decisions made during the developmental and validation processes for this model are broadly based on contributions from an extensive review of the literature and the analysis of spatial data within the select study areas using a GIS.

This model of risk was developed from a four-step process:

- (1) Define the range of variables associated with risk for each factor based on the evaluation and classification of data extracted from the study areas.
- (2) Identify and define the level of risk among US counties based on the calculated mean of their risk factor score.
- (3) Identify and define the level of incidence among US counties based on the mean incidence of human WNND from 2002 to 2008.
- (4) Validate the model based on a comparison of the level of predicted risk with level of incidence.

#### **STEP 1: DEFINE FACTOR VARIABLES**

The zonal statistics application in ESRI ArcInfo was used to extract the maximum, minimum, and mean range of factor variables, or the numeric range of values specific to each dataset, that are associated with each study zone. Each factor, including elevation, land cover, vegetation, and climate, was evaluated separately to identify the variables that are associated with high-risk of WN infection. A factor score ranging from 0 (low importance) to 1 (high importance) was assigned to the variables based on their importance. According to the literature (Melnick 1951; Bernkopf, Levine et al. 1953; Tber 1996), land cover was the most important aspect in defining areas of high incidence; the factor score for land cover was more heavily

weighted than for other factors, ranging from 0 to 3. A US layer for each factor was reclassified based on its factor score.

### Elevation

SRTM data was re-sampled to 1 kilometer using majority rules, to standardize it to the resolution of the other datasets being used. The minimum, maximum and mean elevation was extracted for each study zone. Considering the extracted variables from all study zones combined, the absolute (ABS) maximum, minimum, and mean ranges were calculated (table 3-3). Important vector species for WN virus tend to be more abundant in lower elevations (<1600) (Eisen, Bolling et al. 2008); therefore, to capture the elevations associated with higher risk, the minimum and maximum mean elevations from 0-1516 meters were given a factor risk score of one. All other variables are considered out-of-range and were assigned a factor score of zero.

Table 3-3: Extraction and classification of elevation.

EXTRACTION			
ELEVATION (m)	MIN	MEAN	MAX
MIN	-74	0	33
ABS MEAN	111	295	846
MAX	1126	1516	2510

CLASSIFICATION	
SCORE	ELEVATION (m)
0	<0 & >1516
1.0	0-1516

### Vegetation

The minimum, maximum and mean percentage of tree cover was extracted from each study zone to represent the level of vegetation. Considering all study zones combined, the maximum percentage of tree cover was 13 (table 3-4). The minimum to

Table 3-4: Extraction and classification of vegetation.

EXTRACTION			
VEGETATION (%)	MIN	MEAN	MAX
MIN	1	5	11
ABS MEAN	1	9	13
MAX	12	12	13

CLASSIFICATION	
SCORE	VEGETATION (%)
0	>13
1.0	1-13



maximum range was relatively low; therefore, the percentage of tree cover from 1-13% was assigned a factor risk score of one. Anything above 13% is representative of heavily forested areas, not conducive to mosquito habitat (Haggett 1994; Joy and Clay 2002). These areas are considered out-of-range and were assigned a factor risk score of zero.

## Land Cover

The percentage of each land cover type was extracted from each study zone and the absolute max and mean percentage of each class was evaluated (table 3-5). Cropland, which is associated with heavy irrigation and cultivation, was the most frequent land cover type among all study zones. Given that agriculture is highly

linked to increased WN virus activity

(Melnick 1951; Bernkopf, Levine et al.

1953; Tber 1996), cropland was assigned the

highest factor risk score of three. Ranging

from 10% to 20% of the mean land cover

within the study zones, grassland, wooded

grassland, open shrubland, and bare ground

were assigned a factor risk score of two.

Representing less than 10% of the land

cover within the zonal areas, woodland,

closed shrubland, and urban and built types

were assigned a factor risk score of one.

Densely vegetated forests are not supportive

of the habitat associated with bird-feeding

mosquitoes (Haggett 1994; Joy and Clay

Table 3-5: Extraction and classification of land cover.

EXTRACTION		
LAND COVER TYPE	MAX %	MEAN%
1 = Evergreen Needleleaf Forest	3.76	0.33
2 = Evergreen Broadleaf Forest	1.37	0.06
3 = Dicluous Needleleaf Forest	0.00	0.00
4 = Deciduous Broadleaf Forest	1.53	0.12
5 = Mixed Forest	7.01	0.40
6 = Woodland	43.49	6.54
7 = Wooded Grassland	67.16	10.01
8 = Closed Shrubland	20.38	4.71
9 = Open Shrubland	83.11	10.98
10 = Grassland	43.20	15.33
11 = Cropland	76.11	22.58
12 = Bare Ground	100.00	16.22
13 = Urban & Built	44.41	4.52

CLASSIFICATION		
SCORE	LAND COVER TYPE	MEAN %
0	1-5	<1
1.0	6,8,13	1-10
2.0	7,9,10,12	10-20
3.0	11	>20

2002); therefore, all forests were considered out-of-range and were assigned a factor risk score of zero.

## Temperature

Elevated temperature are important factors when considering the population density and infection rates among mosquitoes (McIntosh, Jupp et al. 1976; Jupp, Blackburn et al. 1986; Dohm and Turell 2001); therefore, the selected layers

Table 3-6: Extraction and classification of temperature.

<b>EXTRACTION &amp; CLASSIFICATION</b>				
<u>LAYER</u>	<u>SCORE</u>	<u>TEMPERATURE (°C)</u>		
		<u>MIN</u>	<u>MAX</u>	
BIO 5	1.0	26	45	
BIO 8	1.0	10.8	26.5	
EPI SSN	1.0	17.6	44.7	

used in the analysis for temperature was derived from three separate layers to capture elevated or maximum values (table 3-6). The first two were bioclimatic variables based on the maximum temperature of the warmest month (BIO 5) and the mean temperature of the wettest quarter (BIO 8). The third layer was computed based on the epidemic season (EPI SSN) associated with each zone. South Africa reported cases from January to March; whereas the outbreak in Romania was reported from August to October. Seasonally, this was the late summer to early fall for both areas; however, the values extracted were based on the three-month epidemic season associated with each zone. The risk value ranges were based on maximum and minimum values extracted from all zones combined. The study areas in the Eastern hemisphere reported relatively moderate temperatures. The maximum monthly temperature (BIO 5) ranged from 26-45°C, the mean temperature during the wettest quarter ranged from 10-27°C, and the three month EPI SSN temperature ranged from 17-45°C respectively for study zones. All values within the ranges were assigned a risk value of one. Using raster calculator, the Boolean layers were multiplied to isolate the locations where all three layers existed, and a risk value of one was assigned to them.

## Precipitation

Excessive precipitation has been associated with epidemics of WN virus (McIntosh, Jupp et al. 1976; Jupp, Blackburn et al. 1986; Dohm and Turell 2001). Some areas have reported periods of drought followed by heavy rainfall (Epstein, Diaz et al. 1998), while others have reported violent

Table 3-7: Extraction and classification of precipitation.

<b>EXTRACTION &amp; CLASSIFICATION</b>			
<u>LAYER</u>	<u>SCORE</u>	<u>PRECIPITATION (mm)</u>	
		<u>MIN</u>	<u>MAX</u>
BIO 12	1.0	3	1186
BIO 13	1.0	1	174
BIO 14	1.0	0	36
BIO 16	1.0	2	486
BIO 17	1.0	0	135
BIO 18	1.0	0	350

storms with above average precipitation (Tber 1996). There were six bioclimatic layers that captured precipitation during the wettest, driest, and warmest time periods including (table 3-7): total annual precipitation (BIO 12), total precipitation of the wettest (BIO 13) and driest month (BIO 14), and total precipitation of the wettest (BIO 16), driest (BIO 17), and warmest (BIO 18) quarter. The ranges were based on absolute maximum and minimum values extracted from all zones combined. All values within the ranges were assigned a factor risk score of one. Using raster calculator, the Boolean layers were multiplied to isolate the locations where all six layers existed in the US and assign them a factor risk score of one.

### **STEP 2: IDENTIFY LEVEL OF RISK**

The mean factor score was extracted to each US county using zonal statistics. Mean risk by county is calculated as the average of the factor scores (factor score sum / 5). The level of risk is based on three categories: low, moderate, and high (table 3-8). Counties with a mean risk value of one had a factor risk sum of five or greater; these counties were considered high risk and were assigned a value of three. Counties with a mean risk value between 0.5 and 0.9 were considered moderate risk and were assigned a value of two. Counties with a mean risk less than 0.5 were considered low risk and were assigned a value of one.

Table 3-8: Reclassification of mean risk and mean incidence of human WN infection.

Layer	Extracted Values	Reclass Values	Description
Mean Risk	<0.5	1	Low
	0.5 - 0.9	2	Moderate
	≥1	3	High
Mean Incidence 2002-2008 per 100K population	<1	1	Low
	1-10	2	Moderate
	>10	3	High

### **STEP 3: IDENTIFY LEVEL OF INCIDENCE**

Due to uncertainties related to surveillance efforts in the early years of WN virus activity, data on human cases from 1999 to 2001 were excluded. The total cases reported between 2002 and 2008 were summed and standardized by the population from 2008 (total cases / total population \* 100K) to calculate the incidence, or the number of humans that become infected per 100,000 people. The level of incidence is also based on natural breaks and assigned to three categories: low, moderate, and high (table 3-8). Counties with a mean incidence of less than one represent low incidence and were assigned a value of one. A mean incidence between one and ten was assigned a value of two, representing moderate incidence. Areas with a mean incidence greater than 10 were considered high-risk and were assigned the value of three.

### **STEP 4: VALIDATION**

The values (1, 2, and 3) that correspond to low, moderate, and high levels for risk defined by the model and actual incidence were compared and classified according to correctness to create four validation maps for a statistical evaluation (table 3-9). Counties with a perfect match between the level of risk and the level of incidence were assigned a value of one. Those that did not match the level of risk with level of incidence were measured to determine the amount of over or under prediction. If the level of risk was greater than the incidence, the area was over-predicted, and the county was assigned a value of two or three based on the over-prediction of

areas by one or two levels respectively. If the level of risk was less than the level of incidence, the county was assigned a value of four or five, based on the under-prediction of areas by one or two levels respectively.

Table 3-9: Classification of counties by overlay of risk and incidence for statistics.

Map	Measure	Description	Legend Value	(Risk, Incidence)
#1	100% Correct	Level Of Risk = Level Of Incidence	1	(1,1) (2,2) (3,3)
	Over Prediction	Level Of Risk > Level Of Incidence	2	(3,2)
			3	(2,1)
			4	(3,1)
			5	(2,3)
	Under Prediction	Level Of Risk < Level Of Incidence	6	(1,2)
			7	(1,3)
#2	Total Correctness	Level Of Risk = Level Of Incidence	1	(1,1) (2,2) (3,3)
		Level Of Risk > Level Of Incidence	2	(3,2) (2,1) (3,1)
		Level Of Risk < Level Of Incidence	3	(2,3) (1,2) (1,3)
#3	Measure of Incorrectness By # of Levels	Level Of Risk = Level Of Incidence	1	(1,1) (2,2) (3,3)
		Level Of Risk <> Level of Incidence By 1	2	(3,2) (2,3) (2,1) (1,2)
		Level Of Risk <> Level of Incidence By 2	3	(3,1) (1,3)
#4	High/Moderate Correctness	Level Of Risk = Level Of Incidence	1	(2,2) (3,3)
		Level Of Risk > Level of Incidence	2	(3,2)
		Level Of Risk < Level of Incidence	3	(1,2) (1,3) (2,3)

### 3.5 RESULTS

#### 3.5.1 FACTOR LAYERS

The five factor layers with their respective values of risk are shown in figure 3-5. The distribution of positive values for land cover is widespread throughout the US; however, the most important types (cropland, value 3; grassland, wooded grassland, open shrubland, and bare ground, value 2) dominate the Midwestern and Western regions, including the valleys of California. This was expected given that these areas are known for agricultural use.

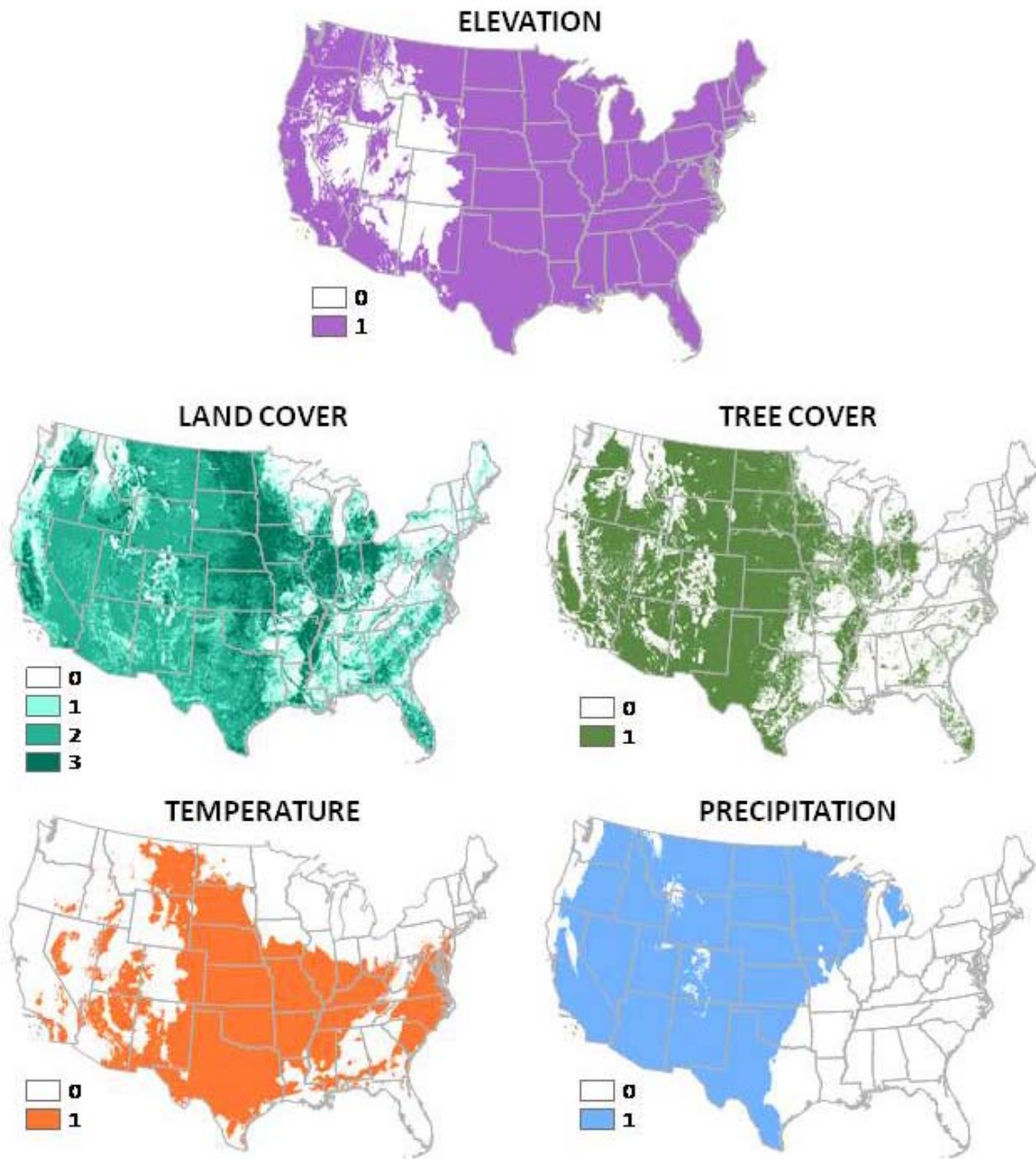


Figure 3-5: Five data layers showing their respective values of risk. Factor layer scores: 0 = Out-of-Range; 1-3 = Weighted factor score.

See tables 3-7 for classification descriptions

Densely vegetative forests block out needed sunlight for mosquito development and they do not support a large population of birds that attract the *Cx.* species. The range of values representing vegetation, or the percentage of tree cover, extracted from the Eastern hemisphere was quite low at 1-13%. The high risk areas based on vegetation, appears to correspond with the high risk areas for the land cover types. Precipitation and temperature clearly support WN virus activity in the midwestern US. The temperatures in the West are mostly below the range, which could be a factor of elevation and coastal influences.

### 3.5.2 LEVEL OF RISK AND INCIDENCE

The mean risk and mean incidence of human WN infection by US county is shown in figure 3-6. High risk areas, developed in this study, cover most of the Midwest and moderate risk extends to the West and parts of the east. High incidence (sum of actual WN incidence from 2001 to 2008) includes the central areas of the West, the Midwest, and the Louisiana and Eastern Texas coast. The distribution of low risk areas corresponds to low incidence in the North, the Western coast, and south along the Appalachian Mountain

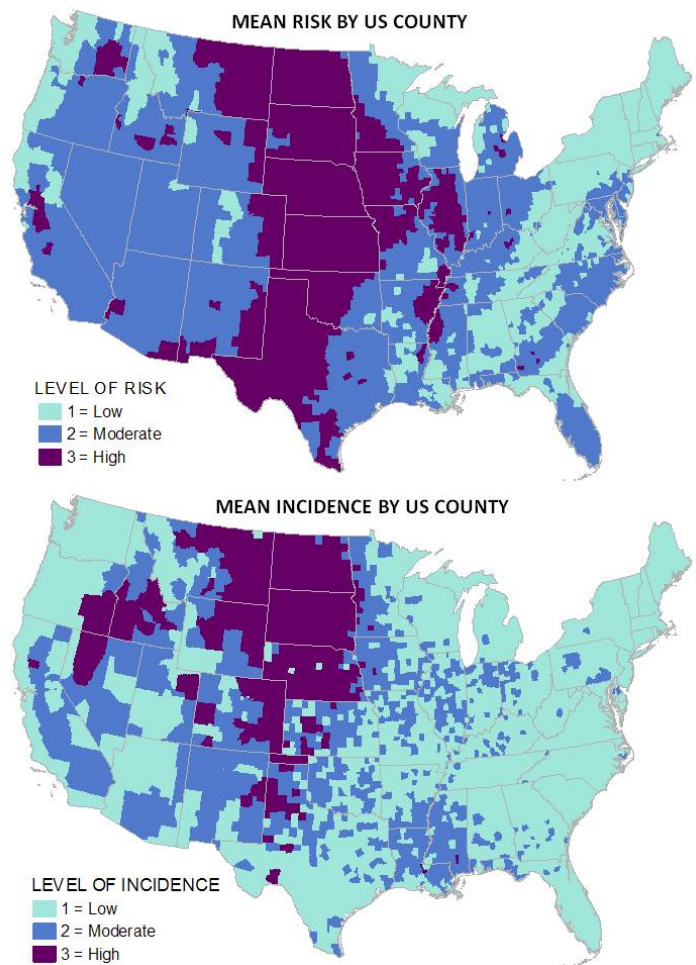


Figure 3-6: Mean Risk and Mean Incidence of human WN infection by US County.

chain. The low incidence in the west could be related to elevation and temperature, which can limit the habitat of mosquito vectors. Moderate risk areas along the Eastern coast and Florida show low incidence. This could be due the dominant existence of *Cx. quinquefasciatus*, which is the least efficient vector of WN virus. The risk throughout most of Texas is moderate to high with very sporadic incidence. This could be due to the lack of reporting, or possible barriers that might exist.

### **3.5.3 VALIDATION**

The validation maps 1 and 2 (figure 3-7) represent the total US counties predicted correctly and incorrectly, and the distribution of over- and under-prediction. The validations map 3 (figure 3-8) represents a measure of over- and under-prediction. The validation map 4 (figure 3-8) represents the correct and incorrect prediction and the distribution of over- and under-prediction of counties with high and moderate incidence.

The counties associated with the values defined by each validation map and the percentages associated with correctness, over and under-prediction, are shown in table 3-10. The percentage of counties correctly predicted (maps 1 and 2) was 45.5% and 50.3% were over-predicted.

Evaluating only counties with a high or moderate level of incidence only (map 4), 60.0% of the counties were predicted correctly and 27.9% were over-predicted.

Based on the scale of this study and the premise that over-prediction is better, 95.8% of the total counties and 87.9% of high to moderate risk counties were predicted correctly or over-predicted. Additionally, 91.1% of counties with high to moderate incidence were predicted as high or moderate risk.



Table 3-10: The percentage of US Counties correctly and incorrectly identified.

MAP	VALUE	RISK & INCIDENCE	COUNTIES		PREDICTION
			COUNT	%	
1	1	(1,1)(2,2)(3,3)	1410	45.5	<u>Total Counties = 3100</u> <b>45.5% = Correct</b> 54.5% = Incorrect Total 46.0% = Off By 1 8.5% = Off By 2 <b>50.3% = Over-Predicted</b> 4.2% = Under-Predicted
	2	(3,2)	301	9.7	
	3	(2,1)	995	32.1	
	4	(3,1)	264	8.5	
	5	(2,3)	43	1.4	
	6	(1,2)	86	6.6	
	7	(1,3)	1	0.03	
2	1	(1,1)(2,2)(3,3)	1410	45.5	<b>95.8% = Correct or Over-Predicted</b>
	2	(3,2)(2,1)(3,1)	1560	50.3	
	3	(2,3)(1,2)(1,3)	130	4.2	
3	1	(1,1)(2,2)(3,3)	1410	45.5	<u>High/Mod Incidence Counties = 1077</u> 60.0% = Correct 27.9% = Over-Predicted 12.1% = Under-Predicted  <b>87.9% = Correct or Over-Predicted</b> <b>91.1% = Predicted as high or mod risk</b>
	2	(3,2)(2,3)(2,1)(1,2)	1425	46.0	
	3	(3,1)(1,3)	265	8.5	
4	1	(2,2)(3,3)	646	60.0	
	2	(3,2)	301	27.9	
	3	(1,2)(1,3)(2,3)	130	12.1	

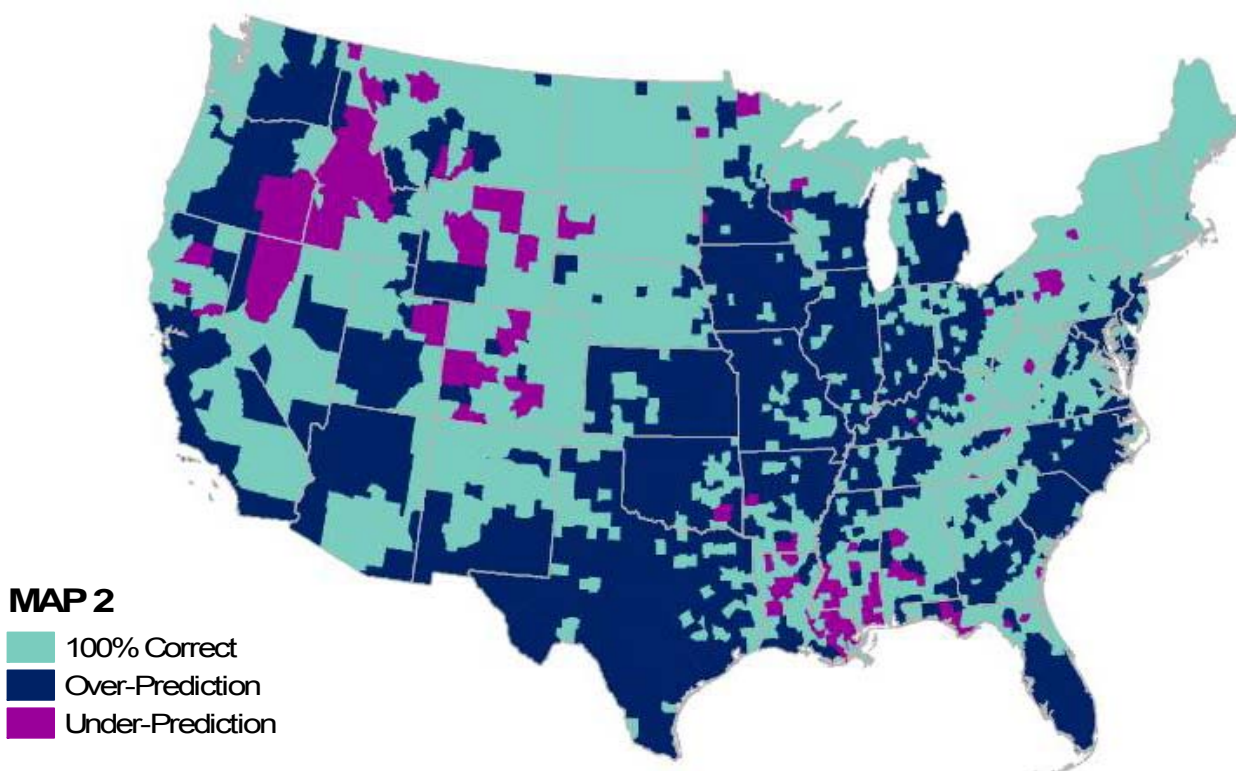
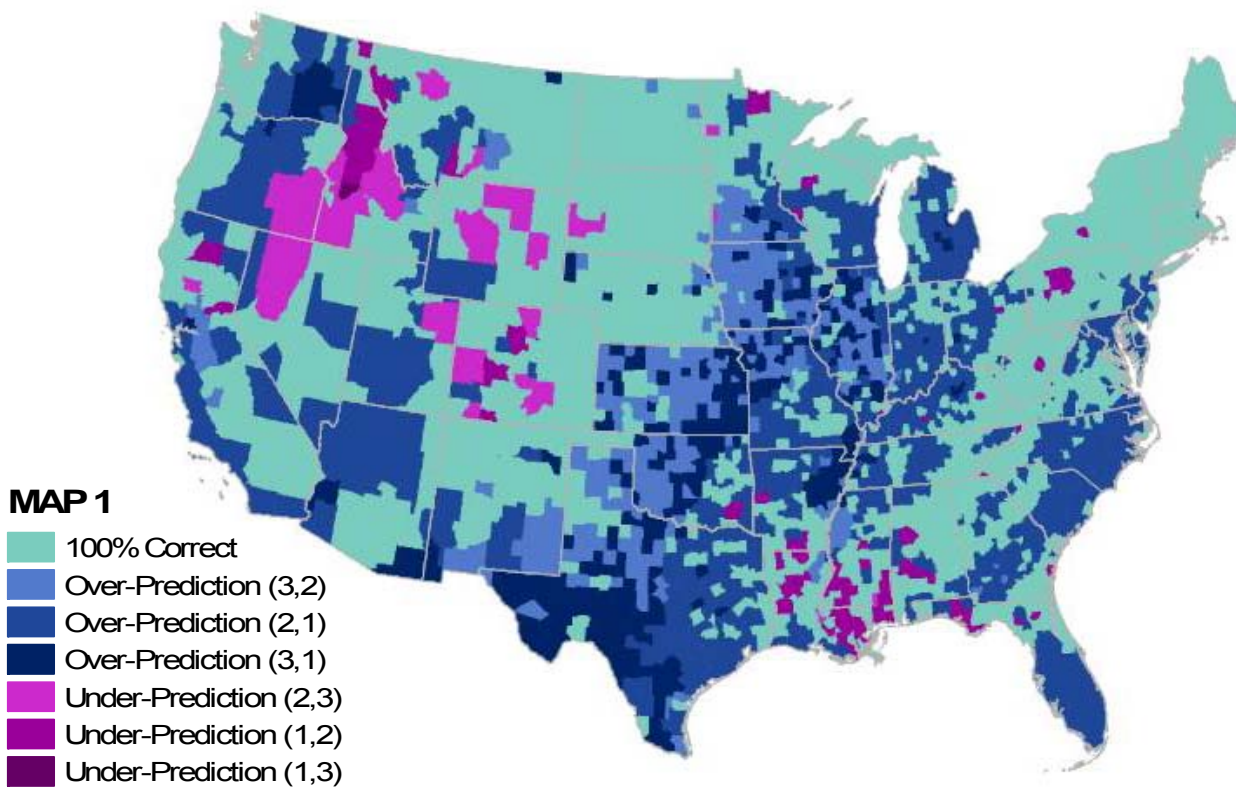


Figure 3-7: Risk model validation maps 1 and 2.

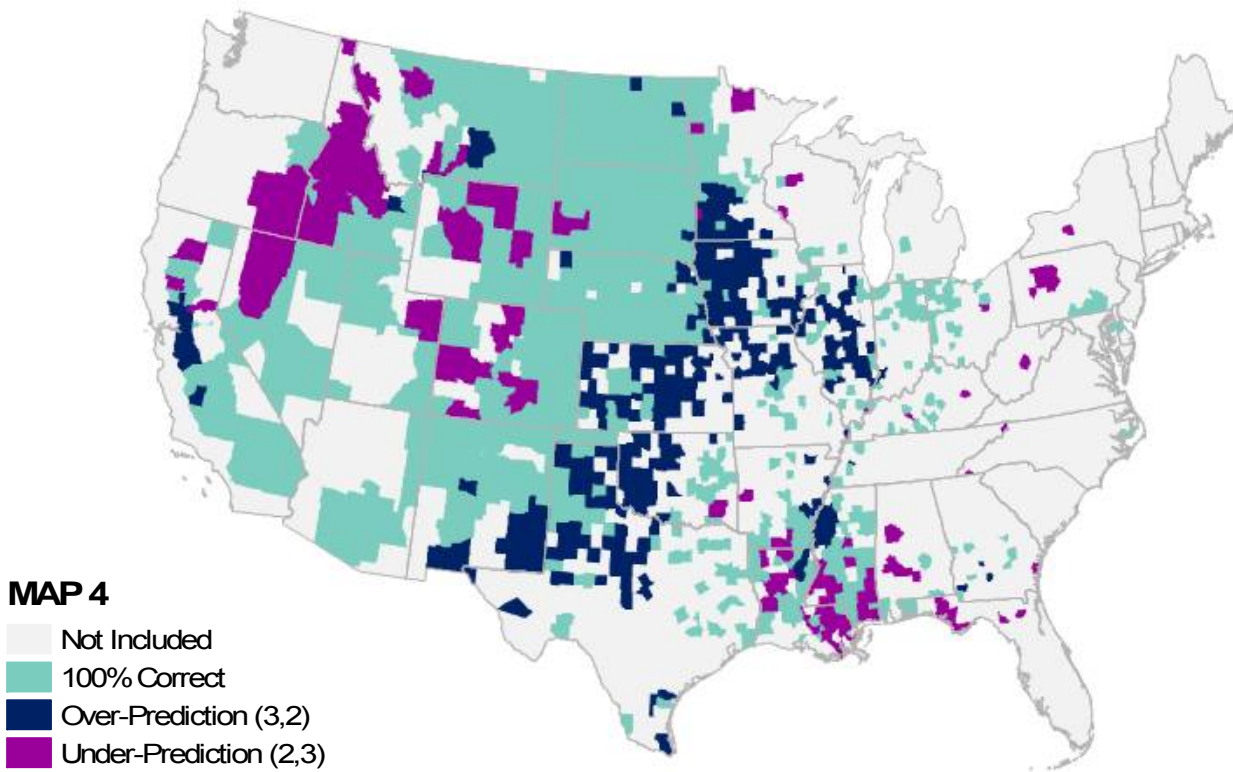
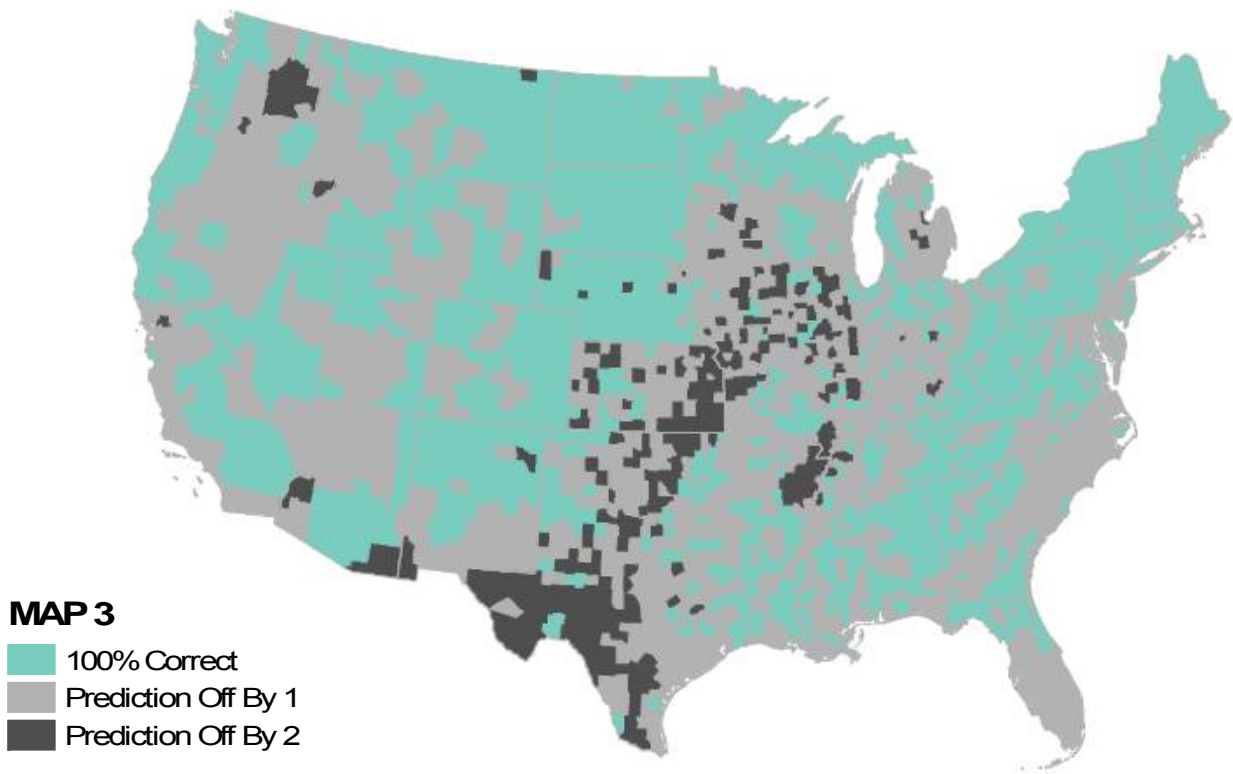


Figure 3-8: Risk model validation maps 3 and 4.

### **3.6 LIMITATIONS**

Although digital data continues to improve in quality and quantity, many dataset did not meet the requirements to be incorporated in this study. Factors that were not considered in this model of risk include: the distribution of mosquito species, factors of human population, land use, water, and urbanization. The incorporation of all important factors that support WN virus activity might produce a more accurate model of risk. The zonal boundaries, which were based on political divisions and digitization, may have influenced the variables associated with risk. There is no way to calculate the amount of uncertainty among these zones without a more collaborative effort from public health officials of the inclusive countries. The accuracy of human case data, provided by the CDC ArboNet surveillance system, is a factor of the level of technology and diagnostics, and the quality of the surveillance efforts of each US County. A process for the collection of human case data needs to be collaborative to standardize the data at the national level.

### **3.7 DISCUSSION**

This study has identified the important ecological factors and processes that are involved in the transmission of WN virus, based on over 60 years of literature and serological studies among endemic and epidemic locations in the Eastern hemisphere. Data extractions from the study zones were evaluated to identify the variables used to define the risk factor scores for the US: topography, land cover, vegetation, and climate. A comparison with human incidence of WNND per 100,000 people confirms this model correctly identifies the US regions in the Midwest and West with the highest risk. These areas include some of the most highly irrigated

areas in the US (Hutson, Barber et al. 2004) and are known to be supportive of a large population of *Cx. tarsalis* mosquitoes, an important vector for WN virus (Turell, O'Guinn et al. 2002).

Attempting to incorporate barriers at the national scale may exclude important areas of risk. Common barriers include temperature and elevation. Given that mosquitoes can appear in ranges from below sea-level to over 3,000 meters (Eldridge 2005), it is unclear how elevation affects mosquito habitat. The range of values associated with elevation extracted from the Eastern hemisphere was included, but that is not to say that mosquitoes don't appear above or below that range. Considering the concepts of landscape epidemiology, WN virus can circulate within localized ecosystems (i.e. the natural nidus). A finer scale analysis would be needed to determine the fragmented mosquito habitats within mountainous regions, and to evaluate the areas that were incorrectly identified by the model of risk for the existence of possible barriers.

Currently, there is no way to measure human susceptibility to infection, which is a combination of factors that represent a person's current state of health. These factors include the presence of existing disease, genetics, and possible immunity to the infectious agent. Until there is a way to quantify human susceptibility, it is up to the individual to determine his or her susceptible risk and to take precautions to prevent exposure to WN virus.

Further investigation is needed to determine the best way of representing temperature as a climatic risk factor for human WN infection. This study incorporated three measures of temperature including maximum annual temperature, mean temperature of the epidemic seasonal months, and maximum temperature of the warmest quarter. Other ways to measure the variance in temperature needs to be explored. More importantly, temperature and precipitation is highly dynamic. These factors need to be monitored on a regular basis and should include the level of humidity, cloud cover, temperature in degree days, and changing vegetation.

### 3.8 CONCLUSION

Disease prediction is a very complex process. The results from this study are only the beginning to a much larger project towards predicting the risk of WN infection and other vector-borne diseases. Public health officials can identify and shift their focus for prevention to high risk areas. Information gathered from field work and the analyses of higher resolution GIS data should be evaluated to a better understanding about the landscape of disease. As quality data continues to become available at a higher resolution, future research will incorporate more detailed description of the landscape of WN infection, and we will become better predictors of disease.

Increasing technology including global communication and the use of Geographic Information Systems (GIS) has presented the opportunity for a collaborative research effort in disease prevention. Working together toward a common goal, entomologists, epidemiologists, researchers, and healthcare workers worldwide can share ideas and present data visually in comparison to tables of calculated results. An online database can serve as a gateway for collecting spatial information. The quality and resolution of available digital data is increasing and becoming readily available; software tools are expanding to offer more utilities that assist in processing, organization, and management of large datasets. Input from multiple sources will increase knowledge about disease transmission; the reliability of disease prediction will grow stronger, decreasing the cost of healthcare and improving the overall human quality of life.

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## APPENDICIES

### Appendix A: Terms in Medical Geography

Agent:	Microorganism that causes disease (bacteria, virus, fungus, or protozoa).
Arbovirus:	Virus spread by arthropods.
Arthropod:	Phylum of invertebrates capable of transmitting disease; includes mosquitoes, flies, ticks, fleas, and lice. transmitting disease.
Ecology:	Scientific study of the distribution and abundance of living organisms, and the interactions among and between organisms and their environment.
Encephalitis:	Inflammation of the brain.
Encephalomyelitis:	Inflammation of the brain and spinal cord.
Endemic:	Present in a place often at low levels.
Epidemic:	Present in a place at above average levels.
Epidemiology:	The scientific study of epidemics and epidemic diseases; especially the factors that influence the incidence, distribution, and control of infectious diseases.
Eradication:	Kill the disease from a geographic location.
Host:	Organism infected by disease agent (people, birds, horses, etc.).
Incidence	The number of new cases diagnosed in a certain defined time.
Meningitis:	Inflammation of the meninges, or the tissue surrounding the brain or spinal cord.
Meningoencephalitis:	Inflammation of the brain, spinal cord, and the meninges.
Natural Nidus	
Pandemic:	An epidemic that spreads to affect an entire region, continent, or the world.
Pathology:	The study of the characteristics, causes and effects of disease.
Prevalence	The total number of people diagnosed in a certain defined time.
Reservoir:	Continual source of possible infection for people.
Vector:	Harbors disease agent and transmits to host.



Appendix B: Vector Species for West Nile Virus.

	USA	EUR	AFR	ASA		USA	EUR	AFR	ASA		USA	EUR	AFR	ASA	
<b>MOSQUITOES</b>					<i>Culex</i>						<i>Ochlerotatus</i>				
<i>Aedes</i>					<i>antennatus</i>			X		<i>atlanticus</i>	X				
<i>aegypti</i>	X		X		<i>coronator</i>	X				<i>atropalpus</i>	X				
<i>africanus</i>			X		<i>decens group</i>			X		<i>canadensis</i>	X				
<i>albocephalus</i>			X		<i>erraticus</i>	X				<i>cantator</i>	X				
<i>albopictus</i>	X				<i>erythrothorax</i>	X				<i>dorsalis</i>	X				
<i>albothorax</i>			X		<i>ethiopicus</i>			X		<i>dupreei</i>	X				
<i>cantans</i>		X			<i>guiarti</i>		X			<i>fitchii</i>	X				
<i>caspius</i>		X			<i>modestus</i>		X		X	<i>fulvus pallens</i>	X				
<i>cinereus</i>	X				<i>neavei</i>			X		<i>grossbecki</i>	X				
<i>circumluteolus</i>			X		<i>nigripalpus</i>	X				<i>infirmatus</i>	X				
<i>excrucians</i>		X			<i>nigripes</i>			X		<i>japonicus</i>	X				
<i>juppi-caballus</i>			X		<i>perexiguus</i>				X	<i>melanimon</i>	X				
<i>madagascarensis</i>			X		<i>perfuscus group</i>			X		<i>nigromaculis</i>	X				
<i>vexans</i>	X		X	X	<i>pipiens</i>	X	X	X	X	<i>provocans</i>	X				
<i>Aedeomyia</i>					<i>pipiens/restuans</i>	X				<i>sollicitans</i>	X				
<i>africana</i>			X		<i>pipiens complex</i>	X				<i>squamiger</i>	X				
<i>Anopheles</i>					<i>poicilipes</i>			X		<i>sticticus</i>	X				
<i>atropos</i>	X				<i>pruina</i>			X		<i>stimulans</i>	X				
<i>barberi</i>	X				<i>quinquefasciatus</i>	X		X	X	<i>taeniorhynchus</i>	X				
<i>brunnipes</i>			X		<i>restuans</i>	X				<i>triseriatus</i>	X				
<i>coustani</i>				X	<i>salinarius</i>	X				<i>trivittatus</i>	X				
<i>crucians</i>	X				<i>scottii</i>			X		<i>Psorophora</i>					
<i>franciscanus</i>	X				<i>species</i>	X		X		<i>ciliata</i>	X				
<i>freeborni</i>	X				<i>stigmatosoma</i>	X				<i>columbiae</i>	X				
<i>hermsi</i>	X				<i>tarsalis</i>	X				<i>ferox</i>	X				
<i>maculipalpis</i>			X		<i>terrants</i>	X				<i>howardii</i>	X				
<i>maculipennis</i>					<i>thelleri</i>			X		<i>Uranotaenia</i>					
<i>punctipennis</i>	X				<i>thriambus</i>	X				<i>sapphirina</i>	X				
<i>quadrimaculatus</i>	X				<i>tritaeniorhynchus</i>			X	X	<b>SOFT TICS</b>					
<i>species</i>			X		<i>univittatus</i>			X	X	<i>Argas</i>					
<i>subpictus</i>				X	<i>vishnui group</i>				X	<i>hermanni</i>			X		
<i>walkeri</i>	X				<i>weschei</i>			X		<i>Ornithodoros</i>					
<i>Coquilleltidia</i>					<i>Deinocerites</i>					<i>capensis</i>		X		X	
<i>metallica</i>			X		<i>cancer</i>	X				<b>HARD TICS</b>					
<i>microannulata</i>			X		<i>Mansonia</i>					<i>Amblyomma</i>					
<i>pertubans</i>	X				<i>titillans</i>	X				<i>variegatum</i>			X		
<i>richiardii</i>		X		X	<i>uniformis</i>			X		<i>Dermacentor</i>					
<i>Culiseta</i>					<i>Mimomyia</i>					<i>marginatus</i>		X			
<i>incidens</i>	X				<i>hispidia</i>			X		<i>Hyalomma</i>					
<i>impatiens</i>	X				<i>lacustris</i>			X		<i>detritum</i>				X	
<i>inornata</i>	X				<i>species</i>			X		<i>marginatum</i>		X		X	
<i>melanura</i>	X				<i>splendens</i>			X		<i>Rhipicephalus</i>					
<i>morsitans</i>	X				<i>Orthopodomyia</i>					<i>muhsamae</i>			X		
					<i>signifera</i>	X				<i>turanicus</i>		X		X	

\*United States of America (USA), Europe (EUR), Africa (AFR), and Asia (ASA)

Appendix C: West Nile Virus Activity in Africa, 1937 to 1997

**WEST NILE VIRUS ACTIVITY IN AFRICA, 1937 to 1997**

COUNTRY	YEAR	SSN	REGION	LOC	MOSQUITO SPECIES		AVIAN		EQUINE			HUMANS				SIGNIFICANCE
					ATB	DS	ATB	DS	ATB	TYPE	CS	DS	ATB	TYPE	CS	
Uganda	1937	DEC	West Nile District NW Uganda	R	NT	NT	0	NT			0	Y	ISO	1c	0	First appearance of WN virus.
Egypt	1950-1951	JUL	Village 30 km N of Cairo Sindbis District N Egypt	R	NT	NT	0	NT			0	70%	ISO	3c	0	In endemic areas: WN Fever is a childhood disease. Antibodies can be found among a large
		JUL-SEP	Egyptian Nile Valley & Delta Sindbis District N Egypt	R	Cx. univittatus (D) Cx. antennatus Cx. pipiens	65%	0	81%	ES	1c	1c	70%	F	1000s 23c age 0-4	0	portion of the adult population. Antibody rates among equine exceed those of humans.
South Africa	1974	eJAN-bJUN	Karoo District W South Africa	R	Cx. univittatus (D) Cx. theileri	53%	0	NT	ES	76c 131s	1c	45-57%	F	18,000s 6c	0	Largest confirmed outbreak of WN fever. First major outbreak among equines.
		mDEC-mAPR	Witwatersrand-Petoria Region E South Africa	R	Cx. univittatus (D) Cx. theileri	Y	0	NT			0	Y	F	5c	0	In endemic areas ... Outbreaks can be sparked by above normal temperature and
Algeria	1994	bAUG-boCT	Tinerkouk Oasis Timmoun District C Sahara SW Algeria	R	NT	NT	0	NT			0	NT	F E	30c 20c	8c	First outbreak of WN encephalitis Africa. First cases and deaths among children.
Morocco	1996	bAUG-eOCT	Kenitra & Larache Provinces NW Morocco	R	NT	NT	1,000's Geese	NT	ES	94c	42c	NT	F	1c	1c	Major outbreak of WN encephalitis among avian and equine in irrigated areas.
Tunisia	1997	bSEP-mDEC	Mahdia & Sfax Regions E Tunisia	R	Cx. pipiens (D)	NT	1,000's Crows	NT			0	90%	MES	173c	8c	First reports of avian deaths in Africa. First outbreak of WN encephalitis in Tunisia.

SSN (Season): b = beginning, m = middle, e = end of month; LOC (locality): U = Urban, R = Rural, AC = Army Camps, CS= Communal Settlement, NH = Nursing Homes;  
 ANTIBODIES: ATB = Antibodies Among Population; TYPE: ISO = Virus Isolation, F = Fever, M = Meningitis, E = Encephalitis, ES = Encephalomyelitis, MES = Meningoencephalitis;  
 CASES: CS = Cases, DS = Deaths, s = suspected; NT = Not Tested;

Appendix D: West Nile Virus Activity in Asia, 1941 to 1998

**WEST NILE VIRUS ACTIVITY IN ASIA, 1941 to 1998**

COUNTRY	YEAR	SSN	REGION	LOC	MOSQUITO		AVIAN		EQUINE			HUMANS			SIGNIFICANCE		
					Species	ATB	DS	ATB	TYPE	CS	DS	ATB	TYPE	CS		DS	
Israel	1941	eJUL- bOCT	Center & Tel-Aviv Districts C Israel	U/R	NT	0	NT					0	NT	F	500s	0	First and largest unconfirmed outbreak of WN fever in Israel.
	1951	eJUL- bOCT	Ma'ayan Tzvi kibbutz Haifa District W Israel	CS	NT	0	NT					0	NT	F M	122c 1c	0	First confirmed outbreak of WN fever among children in endemic area.
	1952	eAUG- bSEP	Coastal Plain N & C Israel	R AC	NT	0	NT					0	NT	F	36s	0	
	1953	eAUG- bSEP	Hadera & Tel-Aviv Districts N & C Israel	U/R AC	NT	0	NT					0	NT	F	255s 40c 1c	0	
	1957	eAUG- bSEP	Hadera City Hadera District C Israel	AC U/R	NT	0	NT					0	NT	F E	299s 1c	0	First appearance of WN encephalitis.
	1980	mAUG	SE of Beer Sheva Negev Desert	NH AC	NT	0	NT					0	NT	F E	33c 16c	4c	First cases and deaths from WN encephalitis among elderly.
	1998	mJUN- mSEP	Eilat kibbutz S Israel	CS	NT	1000's Geese	NT	Y	ES	18	Y	ES	100%	58- 100%		0	First major outbreak among birds and horses in Asia.

SSN (Season): b = beginning, m = middle, e = end of month; LOC (locality): U = Urban, R = Rural, AC = Army Camps, CS = Communal Settlement, NH = Nursing Homes;  
 ANTIBODIES: ATB = Antibodies Among Population; TYPE: ISO = Virus Isolation, F = Fever, M = Meningitis, E = Encephalitis, ES = Encephalomyelitis, MES = Meningoencephalitis;  
 CASES: CS = Cases, DS = Deaths, s = confirmed, s = suspected; NT = Not Tested;

Appendix E: West Nile Virus Activity in Europe, 1958 to 1998

**WEST NILE VIRUS ACTIVITY IN EUROPE, 1958 to 1998**

COUNTRY	YEAR	SSN	REGION	LOC	MOSQUITO		AVIAN		EQUINE				HUMANS				SIGNIFICANCE
					Species	ATB	DS	ATB	TYPE	CS	DS	ATB	TYPE	CS	DS		
Albania	1958			R									Y		0	0	First presence of WN virus in Europe.
France	1962-1965	bAUG- mSEP	Camargue Region Bouches-du-Rhone Department S France	R	Cx. modestus	UKN	0	Y	E	83c	25s 30%		Y	F M	13c 1c	1c	First major outbreak of WN fever in Europe. First major outbreak of WN equine encephalomyelitis.
Romania	1996	mJUL- mOCT	Bucharest and lower Danube valley SE Romania	R				Y						F M E MES	41c 157c 172c 62c	17c	First major urban outbreak, with 61% of patients resided in urban areas. First and largest confirmed outbreak of WN encephalitis.
Italy	1998	mAUG- bOCT	Padule di Fucecchio Tuscany						E	14c	6c 43%						First presence of WN virus in Italy.

SSN (Season): b = beginning, m = middle, e = end of month; LOC (locality): U = Urban, R = Rural, AC = Army Camps, CS= Communal Settlement, NH = Nursing Homes;  
 ANTIBODIES: ATB = Antibodies Among Population; TYPE: ISO = Virus Isolation, F = Fever, M = Meningitis, E = Encephalitis, ES = Encephalomyelitis, MES = Meningoencephalitis;  
 CASES: CS = Cases; DS = Deaths; c = confirmed, s = suspected; NT = Not Tested.