Using Remote Sensing, Ecological Niche Modeling, and Geographic Information Systems for Rift Valley Fever Risk Assessment in the United States

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at George Mason University

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Fall Semester 2009
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DEDICATION

This is dedicated to my wonderful husband Mark and my amazing daughter Piper in recognition of their love, understanding, and support, and to my parents Barbara Kagey and Roger Atkins who have always encouraged me to reach for the stars.

ACKNOWLEDGEMENTS

I would like to thank all the members of my committee for their invaluable mentoring and guidance in writing this dissertation: Dr. Charles Bailey (Director), Dr. Assaf Anyamba Dr. Ken Linthicum, Dr. James Willett, Dr. David Wong and Dr. Cody Edwards.

I am very grateful to my mother Barbara Kagey for sharing her programming expertise. If not for Mom's help, I would still be entering and standardizing mosquito surveillance data.

I would also like to extend my appreciation to Mrs. Penny Masuoka and Dr. Seth Britch for sharing their expertise in using remote sensing data in the ArcGIS and MaxEnt programs, and for their guidance in the doctoral process. I'd also like to thank my fellow GIS student and now PhD, Dr. Michelle Colacicco, for sharing ecological niche modeling problems and solutions.

I could not have done this without the encouragement and help of my husband Mark and my daughter Piper, my family, and my friends.

Thanks go out to the Air Force for giving me the opportunity to complete my doctorate, and to my boss and co-workers for putting up with my "vacation days" to complete this dissertation.

Lastly, I would like to thank all of the people who have provided data for this research:

Mr. Charles Abadam	Suffolk County Mosquito Control
Dr. Jorge Arias	Fairfax Health Department Vector Control
Mr. Randy Buchanan	Henrico County Mosquito Control
Ms. Lane Carr	Henrico County Mosquito Control
Mr. Mitch Burcham	Langley AFB, VA
Ms. Patricia Ferrao	Alexandria Health Department Vector Control
Dr. Aftab Hussain	Arlington Health Department Vector Control
Mr. Muhammad Tauseef Ahmad	Arlington Health Department Vector Control
Mr. Norman Grefe	Norfolk Health Department Vector Control
Ms. Penny Smelser	Norfolk Health Department Vector Control
Dr. Valerie Stallings	Norfolk Health Department
Mr. Marcus Leeper	Newport News Mosquito Control
Mr. Tim DuBois	Newport News Mosquito Control
Mr. Jake Lekan	Alleghany, Covington, Roanoke
Dr. Charles McComb	Gloucester County Mosquito Survey
Ms. Dreda McCreary	Virginia Beach Vector Control
Mr. Ben McLaurin	Prince William County Mosquito Control
Mr. Andrew Lima	Clarke Mosquito Control
Dr. Sally Paulson	VPI&SU Department of Entomology
Dr. Jim Rindfleish	York County Mosquito Control
Ms. Lisa Wagenbrenner	Hampton Mosquito Control
Mr. Jason Williams	Chesapeake Mosquito Control
Mr. Jason Pevear	Chesapeake Mosquito Control
Mr. George Wojcik	Portsmouth Vector Control
Mr. Mike Harrison	Portsmouth Vector Control
Dr. David Gaines	Virginia State Entomologist
Dr. Chris Barker	UC Davis, PhD
Mr. Andrew Fox	United States Department of Agriculture
Mr. Rick Odom	Virginia Department of Agriculture and Consumer Services
Mr. Matt Knox	Virginia Department of Game and Inland Fisheries
Mr. Nelson LaFon	Virginia Department of Game and Inland Fisheries
Mr. Stu Blankenship	Virginia Information Technologies Agency
Mr. Ryan Miller	United States Department of Agriculture
Mr. Ed Pak	National Aeronautics and Space Administration
Dr. Seth Britch	United States Department of Agriculture

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LIST OF ABBREVIATIONS

AUC – Area Under the Curve

AVHRR – Advanced Very High Resolution Radiometer

CDC – Centers for Disease Control

EEE – Eastern equine encephalitis

ELIZA – enzyme-linked-immunosorbent serologic assay

ESRI – Environmental Systems Research Institute.

FIPS – Federal Information Processing Standards

GIS – Geographic Information System

IGBP – International Geosphere-Biosphere

MODIS – Moderate Resolution Imaging Spectroradiometer

NASA – National Aeronautics and Space Adminstration

NASS – National Agricultural Statistics Service

NDVI – Normalized Difference Vegetation Index

NGA – National Geospatial-Intelligence Agency

NOAA – National and Oceanic Atmospheric Administration

OIE - World Organisation for Animal Health

PRISM – Parameter-elevation Regressions on Independent Slopes Model

RS – remote sensing

RT-PCR – reverse transcription-polymerase chain reaction

RVF – Rift Valley fever

SLE – St. Louis encephalitis

SST – Sea Surface Temperature index

VDOT – Virginia Department of Transportation

VEE – Venezuelan equine encephalitis

VGIN – Virginia Geographic Information Network

WEE – Western equine encephalitis

WHO – World Health Organization

WNV – West Nile virus

ABSTRACT

USING REMOTE SENSING, ECOLOGICAL NICHE MODELING, AND GEOGRAPHIC INFORMATION SYSTEMS FOR RIFT VALLEY FEVER RISK ASSESSMENT IN THE UNITED STATES

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The primary goal in this study was to explore remote sensing, ecological niche modeling, and Geographic Information Systems (GIS) as aids in predicting candidate Rift Valley fever (RVF) competent vector abundance and distribution in Virginia, and as means of estimating where risk of establishment in mosquitoes and risk of transmission to human populations would be greatest in Virginia. A second goal in this study was to determine whether the remotely-sensed Normalized Difference Vegetation Index (NDVI) can be used as a proxy variable of local conditions for the development of mosquitoes to predict mosquito species distribution and abundance in Virginia. As part of this study, a mosquito surveillance database was compiled to archive the historical patterns of mosquito species abundance in Virginia. In addition, linkages between mosquito density and local environmental and climatic patterns were spatially and temporally examined.

The present study affirms the potential role of remote sensing imagery for species distribution prediction, and it demonstrates that ecological niche modeling is a valuable predictive tool to analyze the distributions of populations. The MaxEnt ecological niche modeling program was used to model predicted ranges for potential RVF competent vectors in Virginia. The MaxEnt model was shown to be robust, and the candidate RVF competent vector predicted distribution map is presented.

The Normalized Difference Vegetation Index (NDVI) was found to be the most useful environmental-climatic variable to predict mosquito species distribution and abundance in Virginia. However, these results indicate that a more robust prediction is obtained by including other environmental-climatic factors correlated to mosquito densities (e.g., temperature, precipitation, elevation) with NDVI.

The present study demonstrates that remote sensing and GIS can be used with ecological niche and risk modeling methods to estimate risk of virus establishment in mosquitoes and transmission to humans. Maps delineating the geographic areas in Virginia with highest risk for RVF establishment in mosquito populations and RVF disease transmission to human populations were generated in a GIS using human, domestic animal, and white-tailed deer population estimates and the MaxEnt potential RVF competent vector species distribution prediction.

The candidate RVF competent vector predicted distribution and RVF risk maps presented in this study can help vector control agencies and public health officials focus Rift Valley fever surveillance efforts in geographic areas with large co-located populations of potential RVF competent vectors and human, domestic animal, and wildlife hosts.

Keywords

Rift Valley fever, risk assessment, Ecological Niche Modeling, MaxEnt, Geographic Information System, remote sensing, Pearson's Product-Moment Correlation Coefficient, vectors, mosquito distribution, mosquito density, mosquito surveillance, United States, Virginia, domestic animals, white-tailed deer, ArcGIS

CHAPTER 1: INTRODUCTION

1.1 HISTORY OF THE SPREAD OF INFECTIOUS DISEASES

Throughout history expansion in travel and trade has accelerated the spread of infectious diseases. Millions of people throughout Asia, Africa, and Europe were stricken with plague in the 14th to 17th century. In the 19th century, cholera spread from India and resulted in millions of deaths in Asia, Europe, Africa, and North America (WHO 2007a). The introduction of *Aedes aegypti* from West Africa to North America facilitated Yellow fever epidemics in the 19th and early 20th centuries (Roger *et al*. 2006, Tatem *et al*. 2006c, Tatem *et al*. 2006b). In the 1930s transportation expansion led to the introduction of *Anopheles gambiae* in northeastern Brazil and triggered a malaria epidemic with an estimated 16,000 associated deaths before eradication of the vector (Coggeshall 1944, Tatem *et al*. 2006a). More recently, West Nile virus (WNV) spread rapidly in North America after it was introduced in New York City in 1999 (Nash *et al*. 2001; Komar *et al*. 2003; Glaser 2004; Peterson *et al*. 2003a, 2004a; Vorou *et al*. 2007). By summer 2003, WNV had affected more than 8,000 people (Peterson *et al*. 2004a), and its RNA or antigens had been detected in 43 mosquito species in the United States (Rogers and Randolph 2003, CDC 2004a). The rapid spread of West Nile virus (WNV) in the U.S. again demonstrated that exotic pathogens move between continents with increasing ease (Nash *et al*. 2001), and it highlighted the need to

systematically inventory and monitor potential disease vectors and accurately track the risk for emerging diseases.

Today 75% of emerging infectious diseases are zoonotic (Smolinski *et al*. 2003, Vorou *et al*. 2007), diseases transmitted from animals – both wild and domestic – to humans under natural conditions (Mullen and Durden 2002). The arboviruses (arthropod-borne viruses), such as West Nile virus, are a major cause of morbidity and mortality in humans and other animals in tropical and subtropical parts of the world (Smolinski *et al*. 2003) and represent a large proportion of the newly emerged infectious diseases of worldwide concern (Chevalier *et al*. 2004).

1.2 RIFT VALLEY FEVER: CANDIDATE FOR GLOBALIZATION

Another arbovirus with potential to spread worldwide is Rift Valley fever (RVF) virus (Favier *et al*. 2006). RVF, an acute hemorrhagic viral disease first reported among livestock by veterinary officers in Kenya in 1931 (Daubney *et al*. 1931), is associated with abortions and perinatal mortality in livestock (such as sheep, cattle, goats, camels and buffalo) and other domestic animals (Daubney *et al*. 1931, Davies and Martin 2003). Humans can become infected with RVF through bites of mosquito vectors or from exposure to blood or other body fluids of infected animals (e.g., when slaughtering or handling of infected animals or touching contaminated meat during food preparation) (Linthicum *et al*. 1999, CDC 2004b, Bailey 2005). In humans RVF most often results in a mild febrile illness, however, a small percentage of patients (less than 8%) develop encephalitis, retinitis and generalized hemorrhagic syndrome (Meegan and Bailey 1988).

Over the last 40 years numerous RVF outbreaks have occurred in most countries of sub-Saharan Africa as well as Madagascar and Egypt (Meegan 1981, Zeller *et al.* 1997, House *et al.* 1992). Many of these outbreaks have been devastating to farming economies due to the associated livestock losses and prohibited trade. In September 2000, RVF cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent. This outbreak raised concerns that RVF virus may continue to spread to areas with a variety of ecological conditions that were previously uninfected with the virus (Jupp *et al.* 2002, Anyamba *et al.* 2006, Bird *et al*. 2007, Evans *et al*. 2007, WHO 2007b).

1.3 Factors Contributing To The Emergence and Establishment

Despite advances in infectious disease ecology, the factors that contribute to the emergence and establishment of RVF and other emergent vector-borne diseases are only partly understood (Yamar *et al*. 2005, Favier *et al*. 2006). We do know that factors known to influence vector-borne disease emergence do so indirectly via their relationships with elements of the transmission cycle (vertebrate host, vectors, and virus) (Clements *et al*. 2006), and these factors are closely related to environmental conditions and often present simultaneously or sequentially (Wilson 1995, Brownstein *et al*. 2002, Yamar *et al*. 2005).

Factors known to contribute to emergence and establishment of vector-borne diseases include international travel and trade (e.g., SARS near pandemic in 2003), microbial adaptation and change (e.g., the evolution noted in Avian influenza A, H5N1) (Pherez *et al*. 2007, Vorou *et al*. 2007), human susceptibility to infection, climatic variations and changes, economic development and land use (e.g., increasing proximity of human and animal

populations), human demographics and behavior (e.g., urbanization, tourism, and outdoor activities), technology and industry, breakdown of public health measures (e.g., lack of potable water, unsanitary conditions, and poor hygiene), poverty and social inequality, war and famine, and lack of political will (Wilson 1995, Smolinski *et al*. 2003, Vorou *et al*. 2007). The combination of these factors can create an environment in which vector-borne diseases such as Rift Valley fever can emerge and become established in new geographic regions.

1.4 PREDICTING AND DETECTING RIFT VALLEY FEVER OUTBREAKS

Since spatial and temporal changes in climatic variables can change the prevalence and distribution of the vectors that transmit RVF virus (Longstreth and Wiseman 1989, Randolph *et al*. 2002), RVF research has focused on identifying and mapping the geographic distribution of vector species and assessing the environmental factors associated with the vectors' habitat (e.g., climate conditions, geologic characteristics, topographic features, and competition with other species). These data in conjunction with remotely-sensed vegetation measurements (e.g., Normalized Difference Vegetation Index) and associated climate data sets (e.g., sea surface temperatures, rainfall measurements, and satellite derived cloudiness indices) have been used to study the conditions that give rise to increases in mosquito populations that spread RVF virus, and subsequently, the spatial and temporal distribution of the disease in Africa (Longstreth and Wiseman 1989, Linthicum *et al*. 1987, Anyamba *et al.* 2001). The 2006-2007 RVF outbreak in Kenya was the first RVF outbreak successfully predicted as a result of such studies (Kaplan 2007, Linthicum *et al*. 2007, Anyamba *et al.* 2009).

1.5 SHOULD THE U.S. BE CONCERNED ABOUT RIFT VALLEY FEVER?

The potential for Rift Valley fever to extend beyond its known historic geographic boundaries, as demonstrated by the RVF emergence in Saudi Arabia and Yemen in 2000, has been a concern for many years (Daubney *et al*. 1931, Gargan *et al*. 1988, Linthicum *et al*. 2007). The United States is recognizably among the areas receptive to emergence and establishment of the RVF virus. Regardless of the pathway the virus may follow to enter the U.S. (e.g., entry of infected airline passengers originating from RVF endemic countries, mechanical transport of RVF virus-infected vectors, and smuggling of live virus), all of the components (vector, host, and environment) necessary to sustain a Rift Valley fever outbreak are present in this country (Kasari *et al.* 2008).

Over thirty African mosquito species have been implicated as vectors of RVF virus (Meegan and Bailey 1988). Many U.S. mosquitoes of the same genera (*Aedes*, *Culex*, and *Eretmapodites*) are capable of transmitting the RVF virus (Turell and Bailey 1987, Turell and Perkins 1990, Turell *et al*. 1990, Turell and Rossi 1991, Traore-Lamizana *et al*. 2001), and climatic conditions are suitable for competent vectors to exist in virtually every region (Gargan *et al*. 1988). During inter-epizootic periods in Africa, transovarial transmission is a likely mechanism for maintenance of RVF virus during times when environmental conditions are unsuitable for active transmission between vector and vertebrate hosts (House *et al.* 1992). In the U.S. there is considerable potential for RVF virus to survive in overwintering mosquito adults or eggs (e.g., *Aedes* spp.) during vector inactivity in the winter and initiate a viral amplification cycle the following spring when wetter and warmer conditions would favor increased breeding of vector species of mosquitoes (Chevalier *et al.* 2004a).

The geographic distribution of potential RVF vectors in the U.S. overlaps that of potential host species (domestic ruminants, wild ruminants, and humans) that can serve as amplifiers for the virus (Kasari *et al.* 2008). The most susceptible animals of U.S. economic interest are sheep, cattle, and goats (Meegan and Bailey 1988, Kasari *et al.* 2008). Retrospective analysis of RVF outbreaks and laboratory studies indicate that RVF virus can infect a wide variety of other animals, including a number of wild ruminant species (Evans *et al.* 2007). White-tailed deer, *Odocoileus virginianus*, if infected during a RVF outbreak in the U.S., could make control or eradication of the virus difficult since this indigenous wild ruminant has a wide geographic distribution in the United States, is found in close proximity to both humans and livestock (Kasari *et al.* 2008), and is immunologically naïve to the RVF virus.

As in RVF endemic regions, in the U.S. RVF virus transmission in humans could result from the bite of infected mosquito vectors or contact with infected vertebrate tissues or aerosols (e.g., food preparation, laboratory work, slaughter of an infected animal or necropsy of an infected animal) (CDC 2008, Britch *et al*. 2007). In addition, humans develop a sufficient viremia to be a source of infection for secondary mosquitoes (House *et al*. 1992) which could contribute to WNV-like spread of the disease within the country if the virus emerged in the United States.

Due to the devastating impacts RVF has had on humans, livestock, and other animals in Africa and the Arabian Peninsula, the World Organization for Animal Health's Office International des Épizooties (OIE) has identified RVF as a major emerging threat (EFSA 2005, OIE 2008b) and has imposed import-export restrictions on countries that have had RVF outbreaks. For instance, ruminants of RVF infected countries with disease (confirmed

RVF transmission) should not be exported unless there is no evidence of RVF on the day of shipment and the animals were vaccinated against RVF at least 21 days prior to shipment, or the animals were held in a mosquito-proof quarantine station for at least 30 days prior to shipment during which they showed no clinical signs of RVF (OIE 2009). Following a RVF outbreak, a country is not considered by the OIE to be a RVF infection free country until it has demonstrated no evidence of RVF infection in humans, animals, or mosquitoes during the four years following a RVF epidemic (Pearson 2000, Linthicum *et al*. 2007, Kasari *et al.* 2008, OIE 2009). The cost of RVF associated trade bans between the Horn of Africa and the Arabian Peninsula has been estimated to be between U.S. $50-75 million per year (Chevalier *et al.* 2004a). The effect of RVF virus in endemic countries suggests that its introduction to the U.S. would result in substantial economic loss and unquestionably have significant effects on the country's public health communities (Linthicum *et al*. 2007).

Because the combined animal and human health impact of RVF virus make it one of the most devastating arboviral pathogens, RVF virus is listed by the Centers for Disease Control and Prevention (CDC) as a select agent that has the potential to pose a severe threat to public health and safety in the U.S. (CDC 2008). In addition, the U.S. Agricultural Bioterrorism Protection Act of 2002 lists RVF virus as a potential bioterrorism agent (USDA 2005).

Whether Rift Valley fever were introduced to the United States intentionally as a bioterrorism agent or naturally due to the convergence of disease emergence factors, presence of a variety of susceptible amplifying hosts and potential competent vectors throughout the diverse ecological zones in the U.S. make establishment of RVF virus in this country a definite possibility. The potential for RVF establishment and the associated impact on

human and animal health, economic loss due to trade bans, and cost of extensive animal/vector control measures make RVF a real threat to the United States.

1.6 RVF Risk Assessment in the United States

Clearly, tracking the emergence, natural introduction or intentional release of this vector-borne disease is a public health and homeland security issue of great importance to the health and economic vitality of the United States (Reisen 2006). As in RVF endemic regions, the risk of RVF emergence in the United States is based on ecological interactions involving host animals, vector arthropods, the virus, and the biological and physical environment (Linthicum *et al*. 2007). Therefore, the integration of these data is necessary to assess the most likely areas for RVF emergence and establishment. By mapping U.S. RVF competent vector abundance and distribution, habitats suitable for competent vectors to thrive, and potential host population distributions, the risk of RVF establishment in mosquito populations and transmission to humans can be estimated.

1.7 Scope and Objectives

Over the last 20 years numerous RVF studies have shown that modeling disease occurrence based on environmental conditions and vector habitat determined by remotely-sensed data can be an effective way of developing a predictive spatial and temporal RVF risk model (Linthicum *et al*. 1987, 1999, 2007; Anyamba *et al.* 2001; Clements *et al*. 2006; Kolivras 2006). These studies and similar studies on Lyme disease (Glass *et al.* 1995, Brownstein *et al*. 2003), hantavirus (Glass *et al*. 2000) and malaria (Hayes *et al*. 1985; Beck *et al*. 1994, 1997; Hay *et al*. 2002; Rogers *et al*. 2002; Zhou *et al*. 2005) have used

geographic information systems (GIS) and remote sensing technologies to retrospectively analyze outbreaks in disease endemic regions as well as to predict future outbreaks.

The objective of this research is to develop a framework for using proven GIS and remote sensing technologies to apply our current understanding of RVF epidemiology and the factors contributing to disease outbreaks in endemic regions to create a RVF predictive risk map for a non-endemic region of the U.S. In addition to vector and environmental factors incorporated in previous disease studies, host (both human and animal) attributes will be included.

Vector-borne disease transmission is unlikely to be uniform over large geographical areas (Rogers and Packer 1993). Since the RVF risk may not be equal across the U.S. and because there is currently no nation-wide mosquito surveillance and disease detection program, studying RVF risk at the regional or state level is a reasonable approach. If RVF were introduced in the U.S., areas in Virginia are hypothesized to be at high risk for disease establishment following an outbreak.

The specific objectives of this study are as follows:

1) To identify potential RVF competent vectors in Virginia.
2) To develop a Virginia mosquito surveillance database.
3) To evaluate the correlation between mosquito density in Virginia and the Normalized Difference Vegetation Index (NDVI) as a proxy for ecological dynamics or other environmental and climatic variables.
4) To evaluate the potential for NDVI to be used as a parameter to predict mosquito distribution.
5) To construct a RVF risk map for Virginia.

1.8 ORGANIZATION OF DISSERTATION

This dissertation consists of six chapters. A general introduction to the threat of emerging infectious diseases in the United States and the goals of this research were presented thus far in this chapter. *Chapter 2* provides a literature review of the RVF virus, history of RVF outbreaks, potential pathways for RVF introduction to the U.S., potential for establishment of RVF in the U.S., use of geographic information systems (GIS) and remote sensing (RS) technology in RVF and other vector-borne disease studies, and use of ecological niche modeling to predict species distribution in un-sampled areas. A description and justification of the study is also provided. *Chapter 3* describes the methodologies employed to accomplish research objectives. *Chapter 4* presents the results from Virginia mosquito surveillance database analyses, mosquito density-environmental/climatic attribute correlation analyses, and interpolation of mosquito data using Normalized Difference Vegetation Index data. The Virginia RVF risk map is presented and described in detail. *Chapters 5* and *6* summarize results and present conclusions of this dissertation work. Future work on RVF and other vector-borne disease mapping is proposed.

CHAPTER 2: BACKGROUND

2.1 RIFT VALLEY FEVER OVERVIEW

To predict and map areas of Virginia at risk for Rift Valley fever virus establishment and transmission to humans, it is necessary to understand the epidemiology of the disease in the regions in which it has historically been recorded (Rogers and Randolph 2003). This includes an understanding of the pathogen, competent vectors, vertebrate hosts and routes of transmission. Also, it is essential is to understand the ecological dynamics in which transmission takes place and the pattern of the disease – both the distribution in space and changes with time. This baseline knowledge is essential to estimate the likelihood of RVF virus to emerge and become established in regions of Virginia.

2.1.1 BIOLOGY OF RIFT VALLEY FEVER VIRUS

The RVF virus is a member of the genus *Phlebovirus* in the family Bunyaviridae (Woods 2002, CDC 2004b). The virus replicates in mosquitoes and in vertebrates quickly and achieves an extremely high concentration primarily in the host liver and other reticuloendothelial cells (Kasari *et al.* 2008). Experiments by Findlay (1931) showed that the virus was stable at 27°C in buffered solutions within the pH range 6.9-7.3 for at least 24h (EFSA 2005). The virus can survive at ambient temperature and also when frozen or lyophilized, but it is sensitive to acidic conditions and is readily inactivated by lipid solvents

(e.g., ether, chloroform), detergents and common disinfectants (e.g., solutions of sodium or calcium hypochlorite) (OIE 2008).

RVF virus is a zoonotic pathogen endemic to Africa (Peters and Linthicum 1994). The susceptibility to and severity of RVF virus infection in numerous vertebrates (e.g., cattle, sheep, goats, camels, rodents, wild ruminants, buffaloes, antelopes, and wildebeest) has been determined during epizootics and in laboratory studies (Table 2.1). Although RVF virus infects a wide range of hosts, including humans, the most significant infections occur in domestic livestock (e.g., sheep, cattle, goats, camels, and buffalo).

Table 2.1. Vertebrates susceptible to RVF virus infection.

Highly fatal[b]	***Moderate disease***[c]	***Mild and/or inapparent disease***[d]	***Refractory to disease***
Lambs[e]	Humans[f]	Cats	Swine
Calves[e]	Sheep	Dogs	Birds
Kids[e]	Goats	Equines	Reptiles
Puppies[e]	Camels	Monkeys	Amphibians
Kittens	Buffalo		
Mice	Rodents[g]		
Hamsters	Cattle		

[a] Information derived from sources cited in text and table modified from Finlay 1931, House *et al.* 1992, and EFSA 2005.
[b] Near 100% mortality, high viremia.
[c] Illness (with some mortality), abortion, and viremia.
[d] Viremia, some illness or no clinical disease.
[e] Less than 1 week of age. In older young, mortality rate is greater in lambs than in kids or calves (20-70%).
[f] Approximately 1% of humans infected with RVF virus develop severe to fatal disease.
[g] Disease may range from mild to highly fatal depending on species and virus strain.

Sheep and Cattle

The most important animal species in RVF epidemics are sheep and cattle. Both sheep and cattle suffer significant mortality (e.g., greater than 90% in lambs and calves less than one week of age) and abortion (virtually 100%) after infection, and they become sufficiently viremic to infect many arthropod vector species (Peters and Linthicum 1994, House *et al.* 1992, EFSA 2005).

Sheep are extremely susceptible to RVF virus. Onset is marked by high fever (40-42 ^{o}C). Significant clinical features in affected lambs, kids, and adult sheep also include listlessness, weakness, anorexia, rapid respiration, excessive salivation, vomiting, fetid diarrhea, and abortion (Daubney *et al.* 1931, House *et al.* 1992). In older lambs and adults, the incubation period is between 24 and 72 hours, and the mortality rate is 20-30% (House *et al.* 1992). The most severe reactions occur in newborn lambs and kids which die within hours of infection, rarely surviving more than 36 hours (Linthicum *et al.* 2008).

Cattle are less severely affected with RVF than sheep. Adult cattle exhibit clinical signs of disease infrequently, but some may develop acute disease with clinical features similar to those of sheep. Frequently abortion is the only manifestation in this species. The mortality rate in native adult non-pregnant cattle does not usually exceed 10 percent (House *et al.* 1992). In calves, jaundice is more frequent and death occurs in 2 to 8 days. Demonstrated mortality rates in calves are generally lower than in lambs and vary widely (20-70%) between outbreaks (Peters and Linthicum 1994, House *et al.* 1992, OIE 2008).

Goats

Goats are generally less severely affected than sheep (e.g., 1977-78 Egyptian outbreak), with much lower morbidity and mortality, fewer abortions, and less severe clinical signs

(Imam *et al.* 1979, Davies and Martin 2003). Abortion in goats and mortality in kids were recorded in Kenya in 1930, the Sudan in 1973, South Africa and Namibia in 1974-75, and in West Africa in 1987 (EFSA 2005). Older kids and goats may develop inapparent, peracute or acute disease (OIE 2008). Table 2.2 summarizes the characteristics of RVF disease in cattle, sheep, and goats.

Table 2.2. Characteristics of RVF disease in cattle, sheep, and goats. *

Feature	***Characteristics***	
	Cattle	**Sheep and Goats**
Incubation Period	1-6 days	Lambs: 12-36 hr Adults: 1-6 days
Clinical signs/symptoms	• Fever of 40°-42°C, anorexia and weakness, listlessness, evident abdominal pain • Calves: depression, icterus, evident abdominal pain • Adults: **frequently asymptomatic**, excessive salivation, diarrhea, fall in milk yield, nasal discharge, **near 100% abortion**	• Fever of 40°-42°C, anorexia and weakness, listlessness, evident abdominal pain • Lambs: evident abdominal pain • Adults: nasal discharge, vomiting, diarrhea, icterus, **abortion rates can reach 100%** • Complications can include hepatitis, cerebral infections, ocular infections
Case-fatality Rate	• Calves: 10%-70% • Adults: <10% in indigenous breeds	• Lambs: 20% (>1 wk of age) to 100% (<1 wk of age) • Adults: 20%-30%

* Adapted from Linthicum et al. 2008.

Camels

Camels do not normally show any clinical signs of RVF infection, however, antibodies to RVF virus have been detected in camels and RVF virus has been isolated from them during epidemics. As in cattle and sheep, high abortion rate (100%) is a common

consequence of the infection in pregnant animals and neonatal mortality may occur in camel foals born during RVF epizootic periods (Davies and Martin 2003).

Equine

Horses develop only low grade viremia following experimental infection (Daubney 1931). During the Egyptian 1977-78 epidemic, a low prevalence of antibody to the virus was detected in the two species (Imam 1981, EFSA 2005).

Swine

Pigs are considered to be refractory (Daubney 1931, Peters and Linthicum 1994). No isolations of RVF virus have been made in pigs during epidemic periods (EFSA 2005). In laboratory studies, pigs are resistant to infection, however, antibodies have been detected in pigs given very high doses of the virus (Scott 1963).

Birds

Poultry and wild birds are not susceptible to RVF virus (Davies and Martin 2003).

Rodents

Many rodents have been shown to be susceptible to RVF virus in the laboratory. Hamsters have been shown to be extremely susceptible to aerosol infection. Antibodies to RVF have been detected in several species of rodents (e.g., African grass rat, *Arvicanthis niloticus*) in Senegal and South Africa (Gora *et al.* 2000, Chevalier *et al.* 2004a). Nevertheless, several studies have suggested that rodents play no role in natural outbreaks of RVF in Africa (Davies 1975, Swanepoel *et al.* 1978, EFSA 2005).

Wildlife

Wildlife species have not manifested any clinical signs of RVF during epizootics of the disease. However, antibody surveys following outbreaks and experimental infection studies

have demonstrated that wild ruminants (buffalo and numerous antelope species), elephants, and rhinoceros (Table 2.3) are among the animals to develop the highest prevalence of antibodies to the virus following inapparent infections (Davies and Martin 2003, EFSA 2005, Evans *et al.* 2007). It is likely that both undetected abortions and mortalities due to RVF infection occur in at least some wildlife species during RVF epidemics (Davies and Martin 2003).

Table 2.3. Wildlife species known to develop antibodies against the RVF virus.*

Animal		*Species*
Wild Ruminants	buffalo	African buffalo (*Syncerus caffer*) Asian water buffalo (*Bubalus bubalis*)
	antelope	kongoni (*Alcelaphus buselaphus*) impala (*Aepyceros melampus*) lesser kudu (*Tragelaphus strepsiceros*) Thomson's gazelle (*Gazella thomsonii*) waterbuck (*Kobus ellipsiprymnus*)
Other Wildlife	elephants	African elephants (*Loxodonta africana*)
	rhinoceros	black rhinoceros (*Diceros bicornis*)

* Table adapted from Evans *et al.* 2007.

Humans

Humans with RVF typically have either no symptoms or mild Influenza-like illness with fever, generalized weakness, muscle and joint pain, dizziness, photophobia, anorexia, and sometimes nausea and vomiting (Davis and Martin 2003, CDC 2004b). Recovery usually occurs within 4-7 days, however, in some cases the disease progresses to ocular disease. Other, often fatal, complications include hemorrhagic fever and encephalitis (which can lead to headaches, coma, or seizures). In humans the case mortality rate is generally low

(approximately 1%), but full recovery may be protracted and long-term ocular and neurological complications have been reported (FAO 2008). However, in some cases mortality can be as high as approximately 25% when proper public health interventions are not undertaken during an epidemic/epizootic as was the case in Sudan in 2007 (WHO 2007b). Table 2.4 summarizes the characteristics of RVF disease in humans.

Table 2.4. Characteristics of RVF disease in humans. *

Feature	***Characteristics***
Incubation Period	2-6 days
Clinical signs/symptoms	• **Fever** lasting 2-7 days (>90% of cases; often with **mild Influenza-like illness**) • **Retinitis** (up to 10% of cases;1-4 wk after onset of fever) • **Hemorrhagic fever** (<1% of cases; 2-4 days after onset of fever) • **Encephalitis** (<1% of cases; 1-4 wk after onset of fever).
Case-fatality Rate	~ 1%

* Adapted from Linthicum et al. 2008.

2.1.2 DIAGNOSIS AND VACCINATION

The mild influenza-like symptoms in single human cases of RVF can be confused with many viral diseases. However, a RVF epizootic outbreak should be suspected if there is a sudden and widespread onset of many abortions in domestic animals, high neonatal mortality and acute febrile disease with the presence of liver lesions. Cases of disease in people associated with the affected animals also assist in making a tentative RVF diagnosis. Climatic and ecological factors such as the presence of high mosquito populations and/or

flooding of grassland depressions can contribute to provisional RVF diagnoses (Davies and Martin 2003).

There are two types of laboratory tests used to confirm provisional RVF diagnoses. The first is to identify or isolate the RVF virus or antigen. For example, the virus can be isolated via intraperitoneal inoculated mice or hamsters, immunofluorescent or peroxidase staining of tissue culture, simple agar gel immunodiffusion tests using liver or spleen tissue, and immune sera RT-PCR (reverse transcription-polymerase chain reaction) (Davies and Martin 2003, OIE 2008).

The second method to confirm provisional RVF diagnoses is to detect specific antibody to the RVF virus. The presence of RVF specific antibody or IgM can be demonstrated with enzyme-linked-immunosorbent serologic assay (ELISA), microtiter virus-serum neutralization tests in tissue culture, or plaque reduction tests in tissue culture (Davies and Martin 2003, OIE 2008).

No specific treatment exists for Rift Valley fever. In most humans RVF cases, symptoms are mild and are managed with supportive therapy. Both inactivated and live-attenuated vaccines have been developed to help control RVF outbreaks (House *et al.* 1992). Routine vaccination of non-pregnant livestock in Africa is recommended prior to outbreaks, but has been prohibitively expensive, leading to endemicity of RVF in most African countries (Balkhy *et al.* 2003, Davies and Martin 2003, OIE 2008). No vaccine is currently licensed or commercially available for humans or livestock in the United States (WHO 2007b, Britch *et al.* 2007).

2.1.3 GEOGRAPHIC DISTRIBUTION

Since the first major outbreak of RVF was recorded close to Lake Naivasha in Kenya in 1930-1931 (Daubney *et al.* 1931, CDC 2004b), RVF outbreaks in Africa have occurred as far north as Egypt, throughout most of sub-Saharan Africa and as far south as Southern Africa (House *et al.* 1992, Davies and Martin 2003). One of the most notable epizootics of RVF occurred in Kenya in 1950-1951 and resulted in the death of an estimated 100,000 sheep (CDC 2004b). The 1977 RVF outbreak in Egypt resulted in both animal and human cases and is believed to have started due to the importation of RVF virus infected domestic animals from Sudan (Gad *et al.* 1986, Peters and Linthicum 1994). In 1987 transmission of the RVF virus to humans in West Africa (Senegal, Mauritania) was linked to the altered interactions between humans and mosquitoes that resulted from flooding of the lower Senegal River during construction of the Senegal River dam project (CDC 2004b). In 1997-1998 a RVF outbreak in East Africa affected 89,000 people and caused over 400 deaths (Gerdes 2004). A severe form of the disease was seen in Mauritania (1998) where many thousands of people became sick, 200 people died, and abortion losses in livestock were heavy (CDC 2004b, Gerdes 2004). The 2000 outbreak in Saudia Arabia and Yemen was particularly alarming as this was the first time RVF virus was detected outside the African continent (Ahmad 2000, Jupp *et al.* 2002, Anyamba *et al.* 2006), and it demonstrated the potential for the virus to spread with devastating consequences to previously uninfected areas with a variety of different ecological conditions (e.g., wet and tropical areas such as the Gambia, hot and arid areas such as Yemen, irrigated regions such as the Senegal River valley) (Chevalier *et al.* 2004a). As in other Rift Valley fever outbreaks, the November 2006 outbreak in the horn of Africa began after several months of heavy rains that caused floods

and created mosquito breeding habitats. In just four months, 155 people had died, and the outbreak had forced the closure of livestock markets in Kenya devastating the economy of the region (CDC 2007). From November 2006 through March 2007 RVF outbreaks occurred in Somalia, Tanzania, Sudan, and Kenya (ProMed Mail 2007). In Kenya alone, there were 684 human cases with 155 deaths (Linthicum *et al.* 2008). The most recent cases of clinical disease or infection (without clinical disease) involving domestic ruminant livestock and humans have occurred in Madagascar, South Africa, and Sudan (WHO 2008, OIE 2008, Kasari *et al.* 2008) (Figure 2.1).

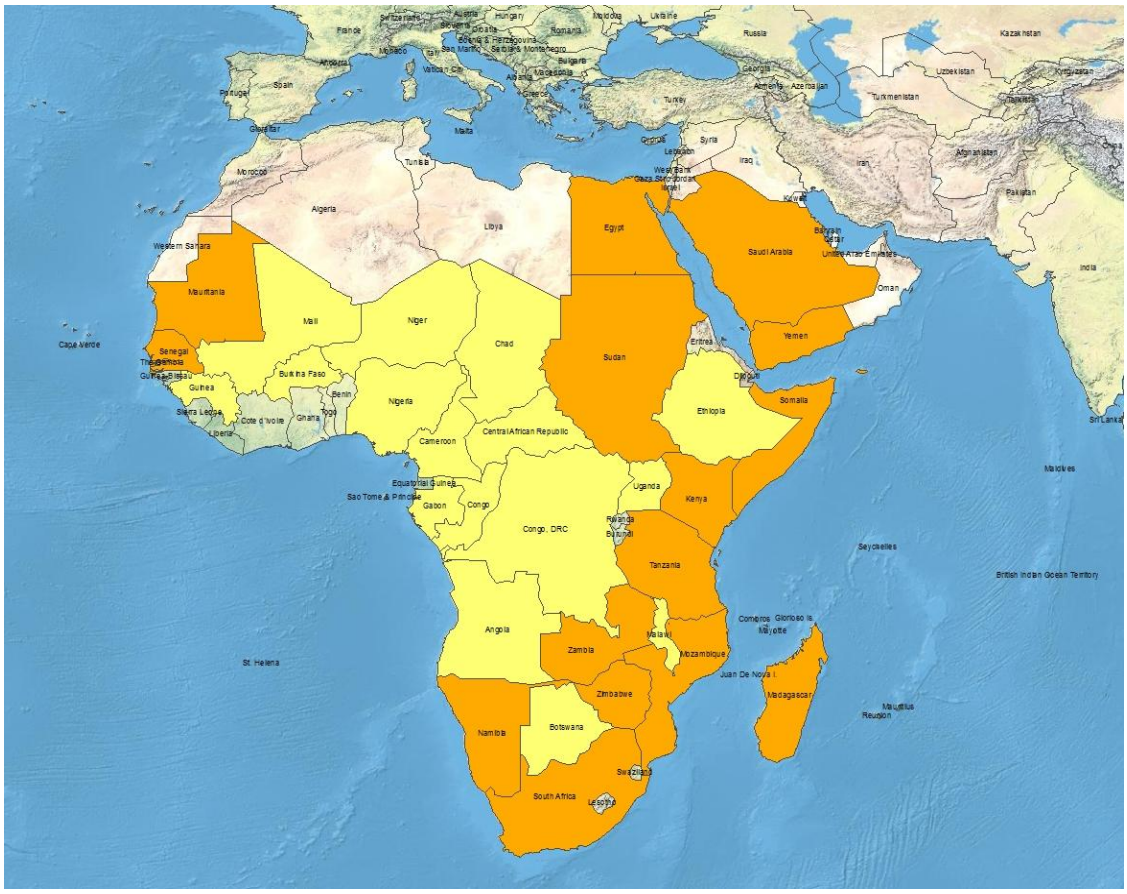

Figure 2.1. Known locations of Rift Valley fever epidemics since 1931. Countries with endemic disease and substantial outbreaks of RVF are shown in orange. Countries known to have some cases, periodic isolation of virus, or serologic evidence of RVF are shown in yellow.

2.1.4 TRANSMISSION AND SPREAD

Modes of Transmission

The mode of RVF virus transmission may be vector-borne, airborne or from direct contact with body fluids of infected animals. Although biting flies (e.g., *Culicoides* spp.)

may transmit the RVF virus mechanically (Hoch *et al.* 1985, House *et* al. 1992, Davies and Martin 2003), mosquitoes are the main RVF vectors transmitting the virus to animals and humans (Meegan and Bailey 1988). Many mosquitoes (e.g., *Aedes, Anopheles, Culex, Eretmapodites*, and *Mansonia*) transmit the RVF virus and are infected naturally (Turell and Bailey 1987, Turell *et al.* 1990, Traore-Lamizana *et al.* 2001, Chevalier *et al.* 2004a). RVF virus is most often transmitted to humans by *Aedes* and *Culex* species of mosquitoes (Linthicum *et al.* 1999, CDC 2004b). Transmission of RVF virus to people working with livestock (e.g., when slaughtering or handling infected animals or touching contaminated meat during the preparation of food or in laboratory facilities) has frequently been an indicator of epizootic RVF virus activity (Davies and Martin 2003). Infection through aerosol transmission of RVF virus has resulted from contact with laboratory specimens containing the virus (Davies and Martin 2003, CDC 2004b), however, there have been no recorded direct human-to-human transmission of RVF virus to date (Kasari *et al.* 2008).

Epizootic and Inter-epizootic Transmission Cycles

The onset and spread of RVF occurs sporadically in time and space. The epidemiology of the disease consists of both epizootic and inter-epizootic transmission cycles (Hoch *et al.* 1985, Meegan and Bailey 1988, House *et al.* 1992). During epizootics, high amounts of RVF virus circulate among mammalian hosts and infected vectors that emerge following persistent heavy rainfall (Davies *et al.* 1985, 1992; Nicholson 1997, Linthicum *et al.* 1999, Woods 2002, Bicout and Sabatier 2004, Chevalier *et al.* 2004a, Diallo *et al.* 2005) associated with El Niño (Linthicum *et al.* 1999, Anyamba *et al.* 2001, Anyamba *et al.* 2009).

The inter-epizootic survival of RVF virus is not clear (Evans *et al.* 2007). In wet or irrigated areas low level virus circulation may persist all year round in permanent *Culex* populations (Chevalier *et al.* 2004a), however, in areas with a dry season there are times when no vectors are present and therefore no transmission occurs. The prevailing hypothesis is that enzootic virus maintenance in these areas depends on transovarial transmission of the virus in floodwater *Aedes* mosquitoes (Davies *et al.* 1985, 1992; Linthicum *et al.* 1985, House *et al.* 1992). As shown in *Ae. mcintoshi*, RVF virus is maintained in the eggs of female floodwater *Aedes* mosquitoes which breed in isolated grassland depressions called dambos (Linthicum *et al.* 1985). The eggs are capable of surviving in dry soil until the next heavy rainfall floods the dambos producing favorable conditions for the eggs to hatch. Subsequently, very large numbers of adult mosquitoes emerge (Linthicum *et al.* 1984; Davies *et al.* 1985, Ba *et al.* 2005, Anyamba *et al.* 2006) and, if infected, transfer the RVF virus to livestock and other animals on which they feed. These vertebrate bloodmeal hosts may become infected and develop a viremia (Linthicum *et al.* 1985, Evans *et al.* 2007). RVF epizootic periods result when waters persist a month or more past the emergence of *Aedes* mosquitoes. This enables secondary vector species (e.g., *Culex* spp.) to breed, generate large populations, feed on animals with high levels of viremia (Linthicum *et al.* 1985, Davis and Martin 2003, Chevalier *et al.* 2004a, Evans *et al.* 2007), and subsequently spread infection to animals beyond the area of the original outbreaks (Linthicum *et al.* 1999, Anyamba *et al.* 2001, Woods 2002, CDC 2004b). Cattle and sheep are the primary amplifiers of the disease (Meegan and Bailey 1988, Longstreth and Wiseman 1989, Kasari *et al.* 2008).

It has also been suggested that reservoir animals (RVF infected rodents or wild ruminants) may be affecting domestic animals in shared grasslands, and thus, maintain the

virus during inter-epizootic periods. Sylvatic (wildlife-mosquito) cycling of RVF virus could maintain the virus at low levels and enable transmission of the virus from wildlife to wildlife and occasionally to livestock (Evans *et al.* 2007). Although Evans *et al.* 2007 found that African wild ruminants do become infected with RVF virus, they concluded "further studies are required to determine whether these animals play a role in the virus maintenance between outbreaks and virus amplification prior to noticeable outbreak" (Figure 2.2).

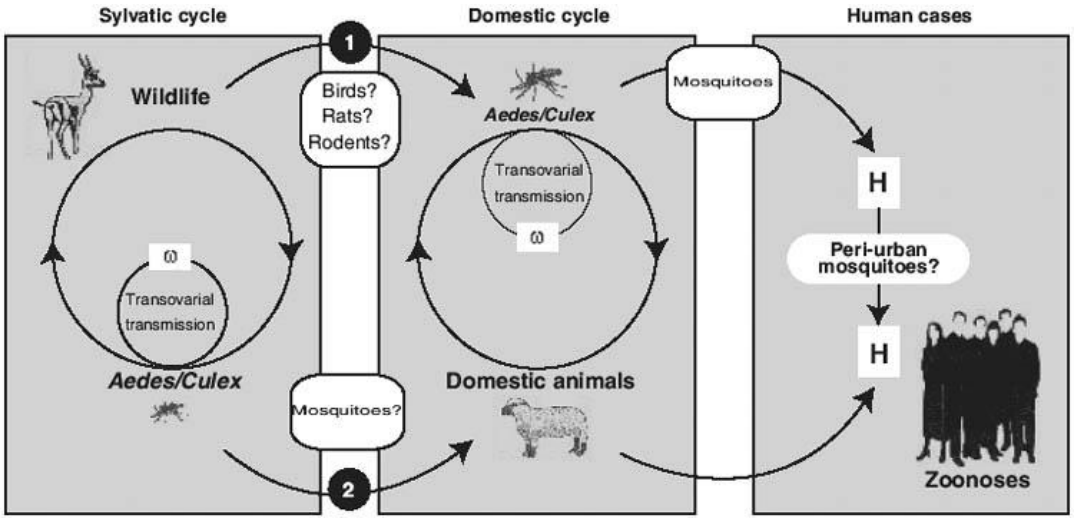

Figure 2.2. RVF virus transmission cycle (from Davies and Martin 2003).

2.2 Potential for Introduction, Establishment, and Spread of RVF in the United States

Rift Valley fever is not endemic to the United States. However, the disease's potentially devastating impacts, continuing occurrence in endemic regions, and expanding ecologic and geographic range promote rising concerns about emergence of RVF virus in the

United States and other non-endemic locations worldwide. Emergence of RVF virus (and other zoonotic arboviruses) in previously uninfected areas such as the U.S. is a multistep process involving initial dispersion and introduction followed by establishment and spread (Tatem *et al.* 2006a, Vorou *et al.* 2007).

2.2.1 Feasible Pathways for Rift Valley fever Dispersion and Introduction

The potential for RVF emergence in the United States initially depends on the existence of viable natural or bioterror related pathways for virus introduction and dispersion/spread into the country.

Natural

The movement of RVF viremic animals through trade represents a threat to both endemic and non-endemic countries that import live animals. For instance, the trade of infected sheep and camels between Sudan and Egypt was believed to be responsible for the 1977 RVF outbreak in Egypt (Chevalier *et al.* 2004a). Peters and Linthicum (1994) noted that if regulations on animal importation are observed, "movement of infected animals is unlikely to be a source of introduction" in non-endemic countries. Still, in a recent evaluation of paths along which RVF virus could enter the U.S. and establish an outbreak of disease in susceptible hosts, Kasari *et al*. 2008 concluded that legal importation of wildlife species is a feasible pathway if quarantine procedures designed to detect infectious diseases at both the country of origin and on entry into the U.S. are circumvented.

Kasari *et al.* 2008 also concluded that air transportation is a feasible pathway for RVF virus entry into the United States. During the last 50 years air travel passenger numbers have

grown by nearly 9% per year (Tatem *et al.* 2006a). Since 2000, despite the impacts of the September 11, 2001 terrorist attacks (9/11), heightened concerns about pandemics, the bankruptcy of airline carriers, and record high fuel prices, travel within the U.S. and internationally has continued to grow (FAA 2008). This expansion increases the potential for introduction of RVF virus and other pathogens to the U.S. via tourists or U.S. citizens viremic with RVF virus contracted during travels to Africa or the Arabian Peninsula (House *et al.* 1992, Ackerman and Giroux 2006).

Globalization of trade and short transport times also make disease containment difficult (Smolinski *et al.* 2003, Gerdes 2004, Ackerman and Giroux 2006). Ships and aircraft transporting commodities (e.g., tires, imported produce) are capable of transporting disease vectors (Wilson 1985). For instance, modern container ships are known to have introduced the Asian tiger mosquito, *Aedes albopictus*, which has been shown in laboratory studies to be a competent vector of 22 arboviruses, including RVF virus (Craven *et al.* 1988, Turell *et al.* 1988, Linthicum *et al.* 2003, Tatem *et al.* 2006c). Mechanical transport of RVF infected vectors trapped in containers on or in the hulls of ships or aircraft transporting commodities is undoubtedly a viable pathway for RVF virus dispersion to and introduction in the U.S. (Kasari *et al.* 2008).

Bioterrorism

Natural outbreaks of RVF illustrate the mass disruption and devastation that could arise from an intentional release (Ackerman and Giroux 2006). Traditionally, humans have been regarded as the primary target for bioterrorism. Human morbidity and mortality resulting from acquisition and proper dissemination of RVF virus could certainly present a

serious public health challenge. Nevertheless, the potential for bioterrorist attacks against agricultural targets (agroterrorism) is increasingly also recognized as a national security threat, particularly since the events of September 11, 2001 and the anthrax attacks that shortly followed (Smolinski *et al.* 2003, Monke 2006).

The U.S. agricultural industry is vulnerable to an intentional RVF attack on livestock in North America. First, a RVF agroterrorist attack would generate panic and fear (of human illness or consumption of infected agricultural products) in a large portion of the population (Lane *et al.* 2001). Second, a RVF agroterrorist attack could severely damage the economy (Ackerman and Giroux 2006). Paarlberg *et al.* (2002) estimated that a Foot and Mouth disease (FMD) outbreak similar to the one that occurred in the United Kingdom in 2001 could result in $14 billion in U.S. farm income losses. A RVF outbreak in the United States has the potential to have a similar effect on the U.S. economy. Containment and eradication of the disease as well as disposal of contaminated products would be costly to individuals, businesses, and governments (Monke 2006). Further harm to the economy would result due to decreased sales associated with public fear of agricultural product consumption and/or decreased food availability (Monke 2006). And U.S. revenues from agricultural product exports (e.g., $60 billion in 2003) would be lost due to OIE trade restrictions associated with RVF (Monke 2006). The cost of RVF-induced trade bans between the Horn of Africa and the Arabian Peninsula has been estimated to be as much as U.S. $75 million per year (Chevalier *et al.* 2004a).

The relative ease for terrorists to procure and disseminate animal pathogens (Ackerman and Giroux 2006) is another reason the U.S. agricultural industry is vulnerable to an intentional RVF attack on livestock. Terrorists could obtain the virus from diseased

animals during RVF outbreaks or collect dormant *Aedes* RVF virus infected eggs from soils in endemic countries. Even though transport and work with RVF virus is closely regulated, terrorists conceivably could obtain the pathogen by illegitimate means from research laboratories (within the United States, in non-endemic European countries, or in endemic countries) that house inventories of the virus (Kortepeter *et al.* 2001).

Many experts agree that smuggling of live RVF virus into the United States is feasible (Kasari *et al.* 2008). As reported by Kasari *et al.* 2008, "there are 152 international airports and 170 sea or river ports of call in the United States, all of which are potential entry pathways for humans who wish to illicitly transport RVF virus." The threat is compounded by the fact that it is difficult to screen passengers and easy to hide the virus.

Once in the United States, terrorists potentially could cause a RVF outbreak by infecting local mosquitoes with the virus, placing dormant *Aedes* RVF virus infected eggs in a suitable environment, introducing an infected animal within livestock herds, or releasing the virus as an aerosol targeted at humans, livestock or wildlife.

2.2.2 Factors that Affect Rift Valley fever Establishment and Spread

Regardless of whether RVF were to emerge in the United States naturally or by intentional means, amplification of the virus would be required for the disease to result in an epidemic, become established, and spread. Virus amplification depends on numerous factors, including prevalence of competent vectors, proximity and susceptibility of amplifying vertebrate hosts, and presence of environmental conditions suitable for virus transmission by competent vectors (House *et al.* 1992, Chevalier *et al.* 2004a).

Prevalence of Competent Vectors

One factor that has enabled RVF to already expand beyond the historically endemic African continent is the unusually large range of competent vectors (Balkhy *et al*. 2003, Gerdes 2004). Vector competence is the ability of a vector species to become infected with an arbovirus and transmit the virus subsequently when feeding on a vertebrate host (Turell 1988, House *et al.* 1992, Mullen and Durden 2002). Intrinsic factors that affect competency of a vector include susceptibility of the vector to oral infection, ability of the virus to disseminate from the midgut to the hemocoel and infect the salivary glands, and secretion of the virus in saliva (Turell 1988, House *et al.* 1992).

Numerous North American mosquito species (including members of the genera *Aedes*, *Anopheles*, *Culex*, and *Eretmapodites*) are competent laboratory vectors of Rift Valley fever virus (Hoch *et al.* 1985, Meegan and Bailey 1988, Gargan *et al.* 1988, Turell *et al.* 1988, House *et al.* 1992, Turell *et al.* 1996, 2008). Nevertheless, the ability to transmit the virus in the laboratory does not mean the species would play a role in RVF establishment and spread in the United States (Turell *et al.* 2005). Other factors such as mosquito population density, host-feeding preference, flight range, and longevity affect how important a particular species would be in transmitting RVF virus (Chevalier *et al.* 2004a, Turell *et al.* 2005).

Proximity and Susceptibility of Vertebrate Hosts

The U.S. has a wide variety of potentially susceptible RVF hosts (including domestic animals, some wildlife species, and humans) that could achieve levels of viremia high enough to infect vectors (House *et al.* 1992, Gerdes 2004). Regardless of the source of virus introduction in the United States, for RVF to become established and spread there must be

sufficient and continued contact between RVF virus-infected mosquitoes and these susceptible amplifying hosts.

Proximity of susceptible hosts to one another and to competent vectors would affect RVF activity. For instance, the rising abundance of container-breeding laboratory competent RVF vectors in urban regions (e.g., *Aedes albopictus*) increases the likelihood of disease transmission to humans (Moore and Mitchell 1997, Moore 1999). Encroachment of human populations on natural animal habitats (Clements *et al.* 2006) has resulted in constant contact between humans and wildlife species. If low levels of RVF virus were maintained due to sylvatic cycling, transmission of the virus from wildlife to wildlife and occasionally to humans and livestock would be likely. Movement of infected hosts (e.g., livestock transportation or human travel) from one area to another would potentially result in spread of the disease to previously uninfected areas. Wide geographic distribution of wildlife (e.g., white-tailed deer and rodents) would make disease containment difficult if these species were to amplify the virus sufficiently to infect mosquitoes that feed on them (Gora *et al.* 2000, Kasari *et al.* 2008). Finally, higher population densities of livestock or wildlife would increase the potential for transmission to individuals within the populations. In addition, higher population densities could also negatively affect the health of individuals, making them more susceptible to RVF virus infection.

Another important factor in host susceptibility to RVF virus may be herd immunity. It has been shown that imported exotic sheep and cattle are highly susceptible to RVF infection compared to indigenous African livestock (Davies and Karstad 1981, Jupp *et al.* 2002, Davies and Martin 2003, Chevalier *et al.* 2004a). It can be assumed that RVF virus

emergence in the U.S. could result in a particularly severe outbreak because domestic animals and wildlife in the U.S. are immunologically naïve to RVF virus.

Suitable Environmental Conditions

The role of environmental elements in the epidemiology of vector-borne diseases such as RVF is well known. Environmental elements such as climate (e.g., temperature, humidity, annual rainfall, intensity of rainfall), hydrology (e.g., proximity to lake/dam, irrigation, accumulated water, proximity to river), and topography (e.g., elevation, land-cover) influence vectorial capacity (House *et al.* 1992, Chevalier *et al.* 2004a, Turell *et al.* 2005, Clements *et al.* 2006). To have high vectorial capacity, which in turn increases the probability of contact between hosts and vectors and the likeliness of RVF virus establishment and spread, competent vectors must be in an environment suitable for vector bioecology (e.g., population dynamics, biting activity and longevity) and virus transmission (Turell *et al.* 2005).

Environmental conditions can affect the ability of mosquitoes to transmit arboviruses such as RVF virus. For instance, the extrinsic incubation (EI) period (the time interval between ingestion of the virus and subsequent transmission by the mosquito) of RVF virus depends on ambient temperature (Brubaker and Turell 1998, Turell *et al.* 1985, Turell 1989, House *et al.* 1992, Diallo *et al.* 2005). In general, studies have consistently shown that the EI period is inversely related to temperature (Turell *et al.* 1985). However, the magnitude of the effect of temperature on both infection and transmission rates appears to vary for different virus-mosquito combinations (Turell *et al.* 1985).

Environmental factors are directly linked to the development, behavior (e.g., biting activity), distribution, abundance and survival rates of vectors (Turell 1989, Kuhn *et al.* 2002, Chevalier *et al.* 2004a). For instance, Alto and Juliano (2001) found that populations of *Ae. albopictus* occurring in regions with relatively high summer temperatures are likely to have high rates of population growth with populations of adults peaking early in the season. *Ae. albopictus* populations occurring in regions with low summer temperatures are likely to experience slow, steady production of adults throughout the season with population size peaking later in the season (Alto and Juliano 2001). For all species, both rainfall and land-use (e.g., irrigation) influence the availability of larval breeding sites and mosquito abundance (Chevalier *et al.* 2004a) as well as mosquito activities (Pherez et al. 2007). Undoubtedly, introduction of RVF virus in the U.S. in the spring, when climatic factors are conducive to mosquito development and survival, would pose a greater threat than an introduction in the late fall when cooler temperatures could kill mosquito populations and potentially end an outbreak (Turell *et al.* 1985, Gargan *et al.* 1988, Turell *et al.* 1988).

Changes in climate (e.g., humidity, rainfall, and temperature) can alter the geographic ranges and life cycles of plants, animals, insects, bacteria, and viruses (Longstreth and Wiseman 1989). Climate changes conducive to vector bioecology in habitats frequented by host species could result in vector population growth and increased disease transmission and/or infectivity (Longstreth and Wiseman 1989). For instance, El Niño/Southern Oscillation (ENSO) related climate anomalies have been shown to impact global tropical precipitation and temperature patterns which ultimately affect vector bioecology and RVF outbreaks (Anyamba *et al.* 2006). Although widespread outbreaks of infectious disease after hurricanes have not been common in the United States, flooding as extensive and persistent

as that experienced August 2005 in New Orleans due to Hurricane Katrina certainly has the potential to create a habitat more conducive to vectors of disease, to alter pathogen and vector prevalence and distribution and, consequently, to increase the risk of vector-borne disease outbreaks.

2.3 Control, Prediction, and Early Detection

The difficultly in controlling emerging diseases and predicting illicit importation of the virus make it impossible to prevent RVF virus emergence in the United States. As previously described, outbreaks in RVF virus endemic countries have had severe economic impacts on the agricultural industry due to associated widespread livestock losses (Linthicum *et al.* 1999) and restrictions on the trade of animals and animal products from infected areas (Chevalier *et al.* 2004a). Clearly, U.S. failure to plan for and respond aggressively to RVF emergence could result in devastating consequences. As in RVF endemic countries, U.S. focus on control, prediction, and early detection (GAO 2004) is necessary to limit the potential impact of RVF in this country.

2.3.1 Control

Control of vectors and host movements is necessary to interrupt the epidemiological cycle of RVF virus and thereby lessen the potential impact of an outbreak by lowering disease transmission rates. Effective vector control methods include hormonal inhibitors such as methoprene, widespread use of vehicle or aerial mounted insecticide sprays targeting adult mosquito species, and strategic treatment of mosquito breeding habitats and soils with larvicides and insecticides, respectively (Davies and Martin 2003). Since viremic host

animals could arrive in an uninfected country within the incubation period, movement of animals for trade from enzootic/epizootic areas should be banned during RVF epizootic periods (Davies and Martin 2003).

Also important in controlling disease spread to and among humans is public education to discourage practices that promote transmission. This includes educating the public to avoid direct contact with the blood and body fluids of sick or dead animals unless appropriate levels of personal protection are used and to use personal protection against mosquito bites (e.g., long-sleeved shirts and pants and mosquito repellent).

2.3.2 Prediction and Early Detection

"Detecting a rise in incidence of a specific disease remains the cornerstone of containment of an emerging communicable threat" (Vorou *et al.* 2007). Prediction and early detection of RVF is a prerequisite to rapid response and effective control of Rift Valley fever. Using geographic information system (GIS) and remote sensing technology and ecological niche modeling, areas at high risk of RVF outbreaks can be pre-identified and monitored for environmental conditions that precede outbreaks. This allows for prediction mapping of outbreaks and/or areas at risk and for detection in the earliest stages of an epizootic which enables rapid response and control (and decrease transmission).

Geographic Information Systems and Remote Sensing Technology

A GIS is a computer-based system that combines digital geo-referenced (spatially-related) and descriptive data for mapping and analysis (Brooker *et al.* 2002, Connor *et al.* 1995). One of the main strengths of a GIS is its ability to integrate different types of spatial

and non-spatial data (Brooker *et al.* 2002). Some examples of the types of data overlaid and analyzed using GIS are population data (e.g., census, socio-economic, and animal population data), land-use and public infrastructure data, transportation networks data (e.g., roads and railways), health infrastructure and epidemiological data (e.g., data on mortality, morbidity, disease distribution and healthcare facilities), and environmental and ecological data (e.g., climate and vegetation data) (Kamel *et al.* 2001).

GIS technology can be used to manage and monitor different aspects of disease, from incident tracking to epidemiologic analysis and assessment of risks (Allen and Wong 2006). For example, a GIS can be used to map available epidemiological information and relate it to factors known to influence the distribution of infectious diseases, such as climate and other environmental factors that affect vector bioecology (Brooker *et al.* 2002, Allen and Wong 2006).

The ability to acquire relevant disease-related climatic information has been enhanced by remote sensing. Environmental remote sensing is the science of gathering geographical data, without direct contact with the object of interest, usually by aircraft or satellite sensors (Logicon 1997). Meteorological satellites observe vast areas and cover the earth daily providing detailed global data in geo-referenced, raster format that is easily input into a GIS. Remote sensing data can be used directly (e.g., satellite rainfall estimates), but are often combined to produce indices that are related to ground-based variables relevant to epidemiological events. Among the remote sensing products related to ground-based variables relevant to epidemiological events are sea surface temperature (SST), cold cloud duration (CCD) correlated with rainfall (Hay and Lennon 1999, Rogers and Randolph 2003) and Normalized Difference Vegetation Index (NDVI).

GIS technology and RS data has been used to identify and monitor environmental factors that influence the distribution and abundance of disease vector populations that affect the incidence of diseases (Pope *et al.* 1992, Wood *et al.* 1992, Beck *et al.* 1994, Connor *et al.* 1995, Hay *et al.* 1996, Thomson *et al.* 1996, Dister *et al.* 1997, Brownstein *et al.* 2002) such as Lyme disease (Glass *et al.* 1992, 1995; Brownstein *et al.* 2003; Rodgers and Mather 2006), African trypanosomiasis (Rogers 2000), hantavirus (Glass *et al.* 2000) and malaria (Hayes *et al.* 1985; Beck *et al.* 1994, 1997; Hay *et al.* 2002; Rogers *et al.* 2002; Zhou *et al.* 2005), schistosomiasis (Cross *et al.* 1984, Brooker *et al.* 2001), bovine tuberculosis (Wint *et al.* 2002), WNV (Brownstein *et al.*2002, Allen and Wong 2006) and RVF (Linthicum *et al.* 1987, 1999, 2007; Anyamba *et al.* 2001, 2006; Clements *et al.* 2006).

Applications of Remote Sensing in RVF studies

Because spatial and temporal changes in climatic variables can change the prevalence and distribution of both the RVF virus and the vectors that transmit it (Longstreth and Wiseman 1989, Randolph and Rogers 2002), RVF research since the early 1980s has focused on using remotely-sensed data to identify and monitor the climatic elements that are important determinants of RVF transmission (Linthicum *et al.* 1987, Anyamba *et al.* 2002). Inferred interannual variability in remotely-sensed vegetation measurements (e.g., NDVI) and associated climate data sets (e.g., sea surface temperatures, rainfall measurements, and satellite derived cloudiness indices) have been shown to coincide with historical RVF outbreak patterns in Africa (Longstreth and Wiseman 1989; Linthicum *et al.* 1987, 1999; Anyamba *et al.* 2002).

In East Africa, the occurrence of RVF outbreaks has been correlated to unusually heavy rainfall associated with El Niño-Southern Oscillation (ENSO) anomalies (Davies *et al.* 1985, 1992; Linthicum *et al.* 1999; Anyamba *et al.* 2001, 2006, 2009; Woods 2002). ENSO refers to the coupled large-scale ocean-atmosphere climate phenomenon linked to a periodic warming (El Niño phase) and cooling (La Niña phase) in sea surface temperatures (SST's) across the central and east-central equatorial Pacific (Huggett 2010). These anomalous changes in SSTs have now been recognized to affect the distribution and patterns of rainfall across the global tropics (Ropelewski and Halbert 1987). Changes in SST's influences atmospheric circulation which impacts global precipitation and temperature patterns (Anyamba *et al.* 2006, GEIS 2008). Historical analyses of RVF outbreak patterns in East Africa show that almost all RVF outbreaks have been preceded by above normal SST's in the equatorial eastern Pacific Ocean and the western equatorial Indian Ocean (Linthicum *et al.* 2008).

NDVI, a vegetation index (VI) derived from sets of remotely-sensed data collected by the advanced very high resolution radiometer (AVHRR) sensor on polar-orbiting meteorological satellites of the U.S. National Oceanic and Atmospheric Administration (NOAA), has been used to identify and map regions where conditions are suitable for the development of RVF epizootics (Linthicum *et al.* 1987, 1999; Anyamba *et al.* 2002, 2009).

NDVI measures the photosynthetic capacity of the global biosphere. Leafy green (healthy) vegetation has a high absorption of chlorophyll in the red portion or band of the electromagnetic spectrum and a high reflectance in the near infrared (NIR) band (Tucker 1979). This unique spectral response of vegetation compared to other land surface cover types makes it possible to differentiate vegetation from other surfaces remotely.

NDVI is calculated as a normalized ratio of measured reflectivity in the red and near infrared (NIR) portions of the electromagnetic spectrum: $NDVI = NIR - Red / NIR + Red$ (NASA EO 2008). The NDVI values range from -1.0 to 1.0. Negative values indicate impervious surfaces (e.g., water, clouds or snow). Values close to zero indicate bare soils or spare vegetation. NDVI tends to increase with increases in green leaf biomass (density) or leaf area index; values above 0.2 indicate increasing amounts of green vegetation and can be used to differentiate categories of green vegetation (e.g., forests, croplands, grasslands) (Tarpley *et al.* 1984, Myneni *et al.* 1995).

Since NOAA satellites have gathered global climate data for more than 25 years, long-term NDVI data records exist (NASA EO 2008). The difference between the average NDVI for a particular month of a given year and the average NDVI for the same month over a period of years is called the NDVI anomaly. The NDVI anomaly can be used to characterize the health of vegetation and infer the amount of rainfall or ground moisture in a given area, relative to the norm (Tucker *et al.* 1985).

In addition to being associated with increasing green biomass and photosynthetic activity, NDVI is a useful correlate of rainfall and ground moisture changes (Nicholson *et al.* 1990; Justice *et al.* 1985; Linthicum *et al.* 1987, 1990). Because vegetation growth is limited by water, the relative density of vegetation is a reliable indicator of moisture. There is a near linear relationship between NDVI and precipitation (Tucker *et al.* 1985, Nicholson *et al.* 1990).

Many studies have demonstrated how this relationship can be used to infer ecological parameters associated with RVF viral activity. These studies have linked vector presence and population dynamics to precipitation and green vegetation dynamics (Tucker *et al.* 1985,

Linthicum *et al.* 1987, Linthicum *et al.* 1990, Goetz *et al.* 2000, Anyamba *et al.* 2002, Chevalier *et al.* 2004a). For example, Linthicum *et al.* (1990) showed that anomalous high NDVI values are correlated with heavy rainfall that floods RVF mosquito breeding habitats and results in the emergence and population expansion of primary (*Aedes* spp.) and secondary (*Culex* spp.) RVF vectors.

The presence of RVF vectors and their population dynamics have also been linked to land-cover and land-use patterns which can be remotely sensed (Chevalier *et al.* 2004a). For example, the annual rainfall at Lake Nasser in Egypt is typically low, resulting in minimal amplification of the mosquito population. The high densities of mosquitoes (*Culex* spp.) observed in this area during outbreaks of RVF in 1977 and 1978 have been attributed to local irrigation practices (Chevalier *et al.* 2004a). Remotely-sensed land-use or land-cover data has been used to estimate the distribution of immature and adult mosquito populations and could potentially be used to assess the risk of host-vector contact that can result in disease transmission (Kuhn *et al*. 2002, Sithiprasasna *et al.* 2005a, 2005b).

RVF Forecasting using Remotely-Sensed Data

Because the onset and spread of RVF in Africa are highly episodic and commonly follow periods where variations of vector abundance are correlated with persistent heavy rainfall associated with ENSO (Davies *et al.* 1985, 1992; Linthicum *et al.* 1999, Anyamba *et al.* 2001, Woods 2002, Bicout and Sabatier 2004, Diallo *et al.* 2005), remotely-sensed climate data are routinely monitored and used to flag areas at high risk of RVF activity (Linthicum *et al.* 1990, 1999; Anyamba *et al.* 2002).

In September 2006, the RVF forecast model developed by NASA's Goddard Space Flight Center in collaboration with the Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS) and U.S. Department of Agriculture's Agricultural Research Service (USDA/ARS) showed that there was a high risk that Rift Valley fever would emerge in the Horn of Africa (Anyamba *et al.* 2006, Wkly Epi 20 2007, FAO 2008). The outbreak in Kenya that shortly followed was the first RVF outbreak successfully predicted as a result of a model based on the analysis of combined satellite data sets of sea surface temperatures, cloudiness, rainfall, and vegetation as an indicator of eco-climatic conditions that give rise to increases in mosquito populations that spread the disease (Linthicum *et al.* 2007, Anyamba *et al.* 2006, 2009). Early warning provided by the model enabled control efforts to be implemented in high risk areas at the earliest stages of the RVF epizootic and the impact of RVF disease was reduced compared to the 1997-1998 epizootic/epidemic (Anyamba *et al.* 2009).

Predicting Vector Distribution with Ecological Niche Modeling

Because spatial and temporal changes in mosquito populations affect the prevalence and distribution of vector-borne diseases such as RVF (Longstreth and Wiseman 1989, Randolph *et al.* 2002), identification and mapping of vector distribution is useful to scientists, public health officials, and decision-makers dedicated to predicting and preventing disease outbreaks.

Ecological niche modeling is an analytical tool used to predict the geographic distribution of a species based on variables such as climate conditions, geologic characteristics, topographic features, and competition with other species (Peterson 2003,

Kolivras 2006). Although occurrence maps exist for many species, these maps can present a biased, potentially incomplete picture of species' geographic distributions because they depict the ecological needs and biogeography of species only in areas sampled for the species (Peterson 2004b). Ecological niche modeling can provide inference into un-sampled and under-sampled areas (Peterson 2004b). These models combine points of known occurrence based on surveillance data with spatially continuous geo-registered environmental layers (e.g., elevation, precipitation, temperature, and land-cover) to infer ecological requirements of a species (Sobero´n and Peterson 2005, Hernandez *et al*. 2006). The geographic distribution of a species is then predicted by mapping the area where these ecological requirements are met (Anderson *et al.* 2002, Egbert *et al.* 2002, Peterson *et al.* 2002, Elith *et al.* 2006). For example, Peterson *et al.* (2003) used an ecological niche modeling approach to determine WNV mosquito vector distributions in North America and produced a map of suitability of the landscape for mosquito transmission of the virus.

2.4 APPLYING WHAT WE KNOW TO THE U.S.

RVF and other vector-borne disease studies (e.g., Lyme disease, hantavirus, and malaria) have forecasted outbreaks and produced risk maps based on area outbreak data, vector dynamics, and environmental and climatic conditions determined by remotely-sensed data (Hayes *et al*. 1985; Beck *et al*. 1994, 1997; Glass *et al*. 1995, 2000; Linthicum *et al*. 1999, 2007; Anyamba 2001; Hay *et al*. 2002; Rogers *et al*. 2002; Brownstein *et al*. 2003; Zhou *et al*. 2005; Clements *et al*. 2006; and Kolivras 2006). Clearly, for the majority of vector-borne diseases the relationship between environmental (predictor) variables and the presence or absence of disease in endemic regions is well established.

In the U.S. RVF is not present and there is no historical climate precedent for RVF outbreaks. Nevertheless, knowledge of factors contributing to RVF outbreaks in endemic regions can be applied to methods proven successful in vector-borne disease and mosquito distribution predictions to investigate the potential risk of RVF establishment and spread in the United States. Because vector-borne disease transmission is unlikely to be uniform over large geographical areas with an extensive variety of ecological regions (Rogers and Packer 1993) and because there is currently no U.S. nation-wide mosquito surveillance and disease detection program, studying RVF risk at the regional or state level is a reasonable approach. Kasari *et al.* 2008 identified twelve U.S. states (California, Florida, Georgia, Massachusetts, Maryland, Minnesota, New Jersey, New York, Pennsylvania, South Carolina, Texas, and Virginia) at greatest risk for experiencing an outbreak of RVF in their domestic and wild ruminant populations and citizens. If RVF were introduced in the U.S., areas in Virginia were hypothesized to be at high risk for disease establishment following an outbreak.

The contribution of this dissertation is the investigation of NDVI as a parameter for U.S. mosquito species distribution prediction and the construction of a Virginia RVF risk map that is transferable to other non-endemic regions of the United States. The results of this research can be used to develop surveillance plans to strengthen prevention, detection, response and control of RVF and other vector-borne disease in the United States. It can enable decision makers to focus limited resources on areas at high risk of disease transmission and permit efficient implementation of mosquito control, animal quarantine and vaccine strategies to provide early-warning of RVF and potentially reduce the spread and impact of the disease in the United States.

2.5 RESEARCH OBJECTIVES

Based on the literature review provided in the preceding sections, the research objectives of this dissertation are:

1) ***To identify potential RVF competent vectors in Virginia.***

A comprehensive review of RVF vector competency studies will be accomplished. The bioecology of RVF vectors with known distributions in Virginia will be summarized.

2) ***To develop a Virginia mosquito surveillance database.***

Since the presence, distribution and abundance of vectors are critical factors in the risk of spread of any introduced mosquito-borne disease, mosquito surveillance data in sampled areas of Virginia will be analyzed and summarized. The database will aid the state in future studies of mosquito-borne diseases and in allocation of resources to areas with larger mosquito populations and potentially greater vector-borne disease risk.

3) ***To evaluate the correlation between mosquito density in Virginia and the Normalized Difference Vegetation Index (NDVI) and other environmental and climatic attributes***.

As discussed in Section 2.3, NDVI is particularly useful for landscape level study of populations since the index captures the combined effects of several climatic variables (temperature, humidity, elevation, soils, land-use, and precipitation) that influence vegetation and the prevalence and distribution of vectors that transmit disease (Linthicum *et al.* 2007). High NDVI values reflect increases in vegetation greenness or greenness anomalies associated with rainfall and warmth which affect mosquito populations. Spatial and temporal

changes in mosquito populations in sampled areas of Virginia will be compared with measured environmental and climatic attributes (NDVI, land-cover, elevation, temperature, and precipitation) represented by digital thematic layers in a GIS.

4) ***To evaluate the potential for NDVI to be used as a parameter to predict mosquito distribution.***

To generate predictions of mosquito distributions over wide areas, it is rarely practical to carry out new mosquito surveys, as these would have to cover the whole region described by the species distribution map. As a result, satellite remote-sensing data (e.g., NDVI) and possibly other environmental and climatic geographic data will be used in conjunction with mosquito surveillance data to identify habitats within Virginia with favorable conditions for these vectors and to construct a species distribution prediction map of mosquitoes suspected to be capable of transmitting the RVF virus in Virginia.

5) ***To construct a Virginia RVF risk map.***

Vector-borne disease risk generally coincides spatially with high densities of host species in areas with environmental conditions conducive to vectors, virus maintenance and virus transmission. If RVF emerges in the U.S., the highest risk of RVF incidence, establishment, and spread would be in areas of highest probable host and competent vector interactions with environmental conditions suitable for virus transmission (Bicout and Sabatier 2004). Intersections of these disease factors will be depicted on a spatial GIS map as areas with higher risk of a RVF outbreak, subsequent establishment, and transmission to humans.

CHAPTER 3: METHODS

The following methods were used to identify potential RVF competent vectors in Virginia, develop a Virginia mosquito surveillance database, predict mosquito distribution based on historical mosquito surveillance and environmental data, and construct a Virginia RVF risk map.

3.1 STUDY AREA

3.1.1 SELECTION METHODOLOGY

Kasari *et al.* 2008 identified twelve U.S. states (California, Florida, Georgia, Massachusetts, Maryland, Minnesota, New Jersey, New York, Pennsylvania, South Carolina, Texas, and Virginia) at greatest risk for experiencing an outbreak of RVF in their domestic and wild ruminant populations and citizens. Although some states (e.g., California and Texas) have larger populations of RVF susceptible domestic animals and would likely suffer greater economic loss with a RVF outbreak, the Commonwealth of Virginia was selected for this study for the following reasons: proximity to the Nation's capital (e.g., potential for human-focused bioterrorism), existence of viable pathways for natural introduction (e.g., presence of ports, citizens, and tourists traveling to and from endemic countries), historically large and growing human populations (e.g., Northern Virginia), presence of potential wildlife

hosts that are widely dispersed throughout the state and abundant in metropolitan areas (e.g., white-tailed deer), presence of domestic animals susceptible to RVF virus (e.g., cattle, sheep, and goats), suitable climate and habitat for RVF competent vectors, presence of RVF competent mosquitoes, and availability of mosquito surveillance data.

3.1.2 Study Area Description

Commonwealth of Virginia

Virginia has an area of 42,774 square miles (110,784 km^2) making it the thirty-fifth largest state by area (National Geographic 2008). The mean elevation of the state is 950 feet above sea level. Geographically and geologically, Virginia is divided into five regions from east to west: Coastal Plain, Piedmont, Blue Ridge Mountains, Ridge and Valley, Cumberland Plateau (Bingham 1991) (Figure 3.1).

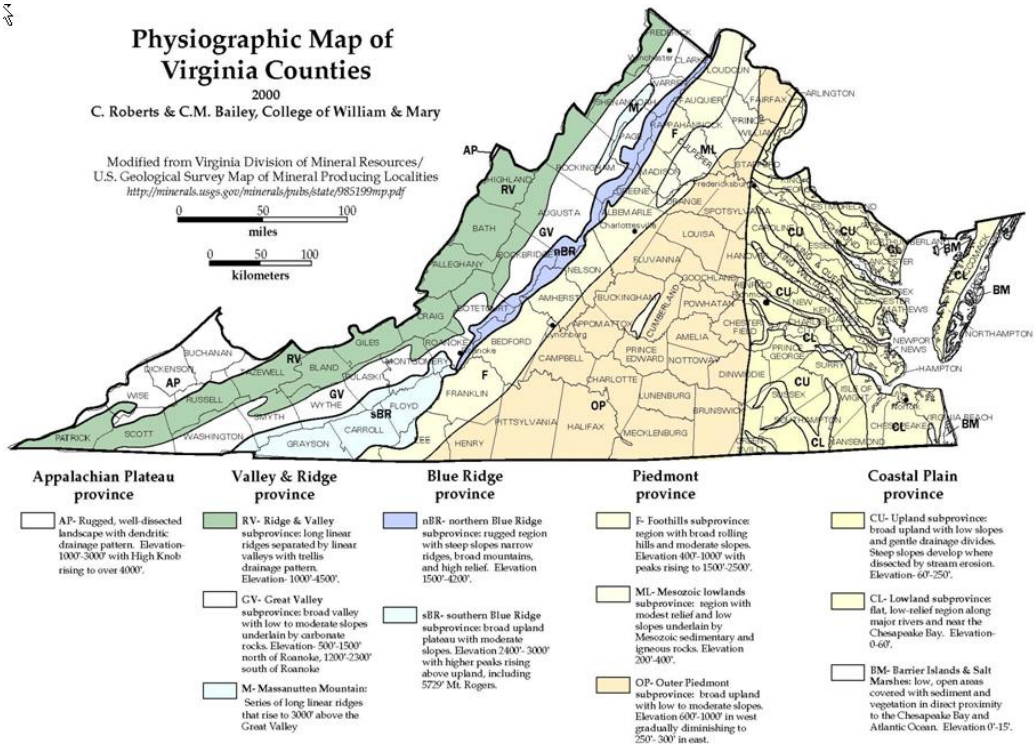

Figure 3.1 Physiographic Map of Virginia Counties (Roberts and Bailey 2000)

The Atlantic Coastal Plain is an area of lowlands running north-south from the Atlantic coast to the fall line, about 100 miles inland. It includes the Eastern Shore and major estuaries which enter the Chesapeake Bay, and it is covered with salt marshes and swamps. It is often called the Tidewater because of the flow of water up and down the coastal inlets and bays as the tide moves in and out. The Piedmont, Virginia's largest geographical land region, lies to the west of the Tidewater region. This region gradually slopes upward from elevations of 200 to 300 feet above sea level in the east to 800 to 900 feet above sea level in the west. The rivers and streams of the Piedmont generally flow in a southeasterly direction, breaking into low waterfalls at the "fall line" where the Piedmont meets the Atlantic Coastal Plain. To the west of the Piedmont, lies the Blue Ridge. The Blue Ridge is the main eastern

mountain range of the Appalachian Mountains. Extending southwest to northeast along Virginia's western border is the Appalachian Ridge and Valley Region. This region is a series of valleys divided by mountains and riddled with caverns carved into limestone. Covered with rivers, streams, and forests, the Appalachian Plateau in the far southwestern portion of Virginia averages about 2,000 feet above sea level (Bingham 1991, Netstate 2007).

The climate of Virginia varies. Most of the state east of the Blue Ridge Mountains, as well as the southern part of the Shenandoah Valley, has a humid subtropical climate typical for east coasts of continents between 25 and 45 degrees latitude. The climate in this area is characterized by hot summers and mild wild winters and a fairly uniform distribution of precipitation throughout the year. In the mountainous areas west of the Blue Ridge, the climate becomes humid continental, marked by more variable weather patterns and temperatures (Grymes 2009).

Annual temperature patterns in Virginia are largely a function of latitude, elevation and nearness to the sea. Temperatures in Virginia range from average lows of 26 °F (−3.3 °C) in January to average highs of 86 °F (30 °C) in July. Land near the coast has a lower annual range of temperatures and milder winter temperatures than inland portions of the state. The average annual precipitation in Virginia is 45 inches. The southeastern and southwestern corners of the state receive the most precipitation (approximately 50 inches) each year. Virginia is rich in natural forests (65% of the state), and can grow standard crops such as corn and soybeans or pasture grasses for cattle (Grymes 2009).

Regional Study Areas in Virginia

In 2006[1] Virginia had an estimated population of 7,640,249 (U.S. Bureau of the Census 2000). Of Virginia's eleven Metropolitan Statistical Areas, Northern Virginia, Hampton Roads, and Richmond-Petersburg are the three most populated.

According to the U.S. Bureau of the Census, in 2000 there were 2,055,014 people in **Northern Virginia**, 26.89% of Virginia's estimated population. This estimate included the combined populations of Arlington, Fairfax, Loudoun, and Prince William counties and the independent cities of Alexandria, Falls Church, Fairfax, Manassas, and Manassas Park (U.S. Bureau of the Census 2000). The U.S. Bureau of the Census estimated in 2006 that the population of Northern Virginia had increased to 2,432,823, around 32% of the state's population.

The majority of Northern Virginia is within the Piedmont physiographic province, having wide, rolling hilltops underlain by weathered metamorphic rocks, and dissected into dendritic drainage patterns. Coastal lowlands (flat low-relief land near the Chesapeake Bay and along major rivers with elevation ranging from 0-60') are found east of Interstate-95. Water in this region drains southeasterly into the Potomac River basin and Chesapeake Bay (Roberts and Bailey 2000, Grymes 2009).

Poor drainage of the moderate to low permeability clay soils of Northern Virginia provides favorable breeding habitat for mosquitoes (Allen and Wong 2006). Mosquitoes may survive the moderately mild winters, and blooms may occur throughout the year but particularly between May and October when temperatures and humidity are higher (Allen and Wong 2006).

[1]2006 demographic data was used to correspond with mosquito and environmental data.

The land area of **Hampton Roads** includes dozens of cities, counties and towns on the Virginia Peninsula and in South Hampton Roads with an estimated population in 2006 of 1.5 million (U.S. Bureau of the Census 2000). Hampton Roads is within the Atlantic Coastal Plain physiographic province. The majority of this region of the state is coastal lowlands with elevations from 60'-250'. The western portion of this region is coastal uplands, broad upland with low slopes and gentle drainage divides. A portion of this region is composed of barrier islands and salt marshes (low, open areas covered with sediment and vegetation in direct proximity to the Chesapeake Bay and Atlantic Ocean) (Roberts and Bailey 2000, Grymes 2009).

Suitable habitat (e.g., tidal and freshwater creeks, canals and forested wetlands, ditch networks to allow for agriculture) is available in Hampton Roads for a broad range of mosquito species.

The **Richmond-Petersburg** region is located in a central part of the state. It straddles the fall line, the meeting zone of the Coastal Plain (coastal lowland and upland) and the Piedmont on the James River at Richmond and the Appomattox River at Petersburg (Netstate 2007). The area is composed of four independent cities (listed in order of population): Richmond, Petersburg, Hopewell, and Colonial Heights. The counties within this region include Charles City, Chesterfield, Dinwiddie, Goochland, Hanover, Henrico, New Kent, Powhatan, and Prince George. As of 2006, the Richmond-Petersburg region had a population of 1,081,753 (U.S. Bureau of the Census 2000).

In the Richmond-Petersburg region as well as the Northern Virginia and Hampton Roads regions, the close proximity of good mosquito habitat threaded within and among high

densities of humans creates a landscape conducive to the transmission of mosquito-borne diseases.

3.2 DATA SETS

The major data sets used in this dissertation include Virginia mosquito vector, human demographic, domestic animal, wildlife, elevation, land-cover, Normalized Difference Vegetation Index (NDVI), temperature, precipitation, bioclimatic, and boundary data. The data sets are grouped as vector, host, environmental-climatic, and administrative boundary (Table 3.1) and described below.

Table 3.1. Primary data sets and sources

Data set	*Description*	*Source*
Vector	Virginia mosquito surveillance data	See Table 3.2
Host	Cattle, sheep, and goat density data	USDA Animal and Plant Health Inspection Service (APHIS) Centers for Epidemiology and Animal Health (CEAH) (http://www.aphis.usda.gov/vs/ceah/) From USDA Agricultural Census data published by the National Agricultural Statistics Service (http://www.nass.usda.gov/Census_of_Agriculture)
	Deer population abundance estimates	Virginia Department of Game and Inland Fisheries (http://www.dgif.virginia.gov/)
	Human demographics density data	U.S. Census Bureau (http://www.census.gov/)
Environmental	SRTM digital elevation data (1 km)	Worldclim (http://www.worldclim.org/)
	Land-cover data (1 km)	Boston University Department of Geography Land Cover and Land Use Dynamics (http://www-modis.bu.edu/landcover/)
	AVHRR NDVI data (8km)	NASA (http://www.gsfc.nasa.gov/)
Climate	Monthly minimum temperature (1 km)	PRISM (http://www.prism.oregonstate.edu/)
	Monthly maximum temperature (1 km)	PRISM (http://www.prism.oregonstate.edu/)
	Monthly mean precipitation (1 km)	PRISM (http://www.prism.oregonstate.edu/)
	Average monthly minimum temperature (1 km)	Worldclim (http://www.worldclim.org/)
	Average monthly maximum temperature (1 km)	Worldclim (http://www.worldclim.org/)
	Average monthly mean precipitation (1 km)	Worldclim (http://www.worldclim.org/)
	Bioclim (1 km)	Worldclim (http://www.worldclim.org/)
Administrative Boundary	State and county level boundaries	ESRI Data & Maps CD included with ArcGIS Desktop
	FIPS Codes	U.S. Census Bureau (http://www.census.gov/)

3.2.1 MOSQUITO VECTOR SURVEILLANCE DATA SETS

Virginia mosquito surveillance data for 2000-2006 used in this study was accomplished by mosquito and vector control districts, state public health agencies, and Virginia Polytechnic Institute and State University faculty and students (Table 3.2) throughout Virginia using standard entomological and vector health techniques. The surveillance data was used to produce statewide mosquito abundance and distribution statistics, to evaluate the relationship between mosquito abundance and distribution and environmental/climatic (predictor) variables, to predict mosquito distribution in un-sampled areas of the state, and to construct a Virginia RVF risk map.

Table 3.2. Sources of Virginia mosquito surveillance data.

Sources of Mosquito Surveillance Data
Alexandria Health Department Vector Control
Alleghany, Covington, Roanoke
Arlington Health Department Vector Control
Chesapeake Mosquito Control
Clarke Mosquito Control
Fairfax Health Department Vector Control
Gloucester County Mosquito Survey
Hampton Mosquito Control
Henrico County Mosquito Control
Langley AFB, VA
Newport News Mosquito Control
Norfolk Health Department Vector Control
Portsmouth Vector Control
Prince William County Mosquito Control
Virginia State Entomologist
Suffolk County Mosquito Control
Virginia Beach Vector Control
VPI&SU Department of Entomology
Wise County
York County Mosquito Control

3.2.2 HOST DATA SETS

Human Demographics Data

Humans provide a breeding area for mosquitoes in the form of water vessels in and around the home, as well as a blood meal that could lead to the transmission of RVF virus. Human residential density at the county level was acquired from the 2000 National Census Bureau data (U.S. Bureau of the Census 2000). A map indicating areas of high human density in Virginia, Maryland, and the District of Columbia is shown in Figure 3.2.

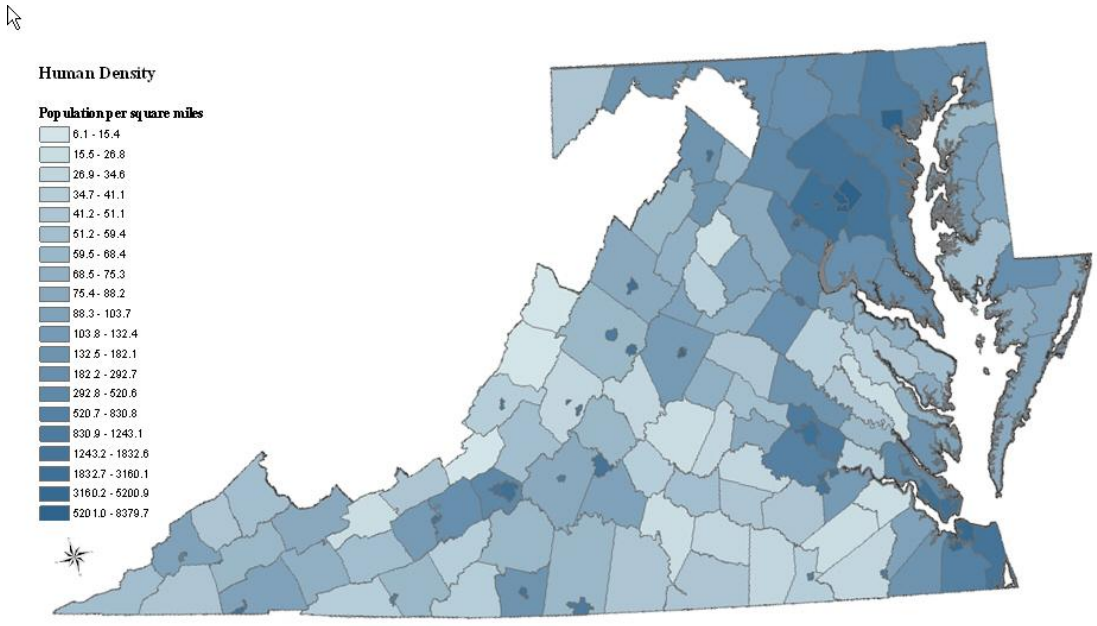

Figure 3.2. Human Density in Virginia. The map shows the density of humans in Virginia as the number of individuals per square mile by county. The data is categorized and displayed in 20 quantiles. Density data for Maryland and the District of Columbia is also shown.

Domestic Animal Data

Virginia domestic animal density data was obtained from the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Centers for Epidemiology and Animal Health (CEAH). USDA/APHIS/CEAH compiled the 2002 USDA Agricultural Census data published by the National Agricultural Statistics Service (NASS 2002). The "NASS data" include both livestock inventory and sales data by facility at the county level for the major U.S. livestock species (cattle, swine, sheep and goats). The NASS Virginia cattle, sheep, and goat inventory data were used in this study as an indicative measure of the animals' density in the state (Figure 3.3-3.5).

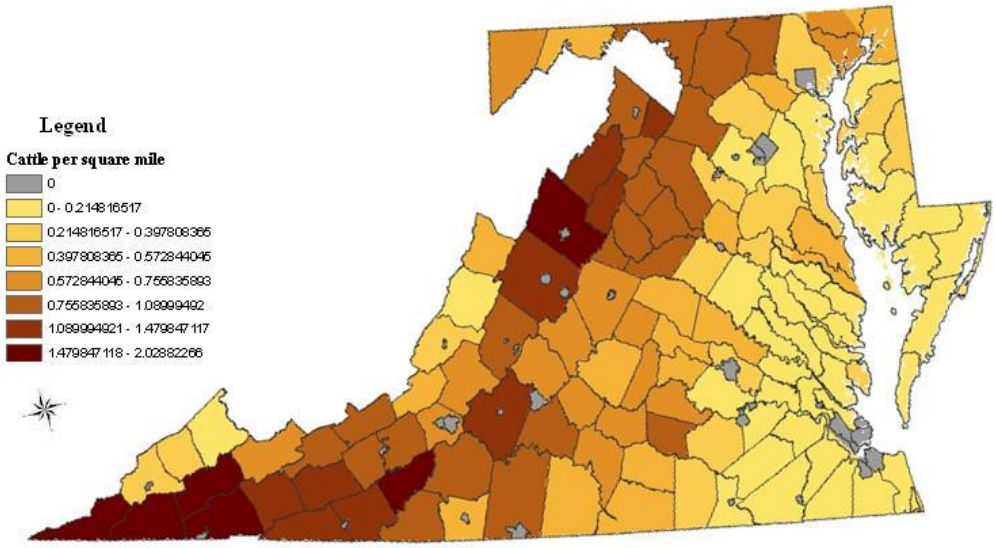

Figure 3.3. Cattle Density in Virginia. The map shows the density of cattle in Virginia as the number of cattle per square mile by county. The data is categorized and displayed in 8 quantiles. Density data for Maryland and the District of Columbia is also shown.

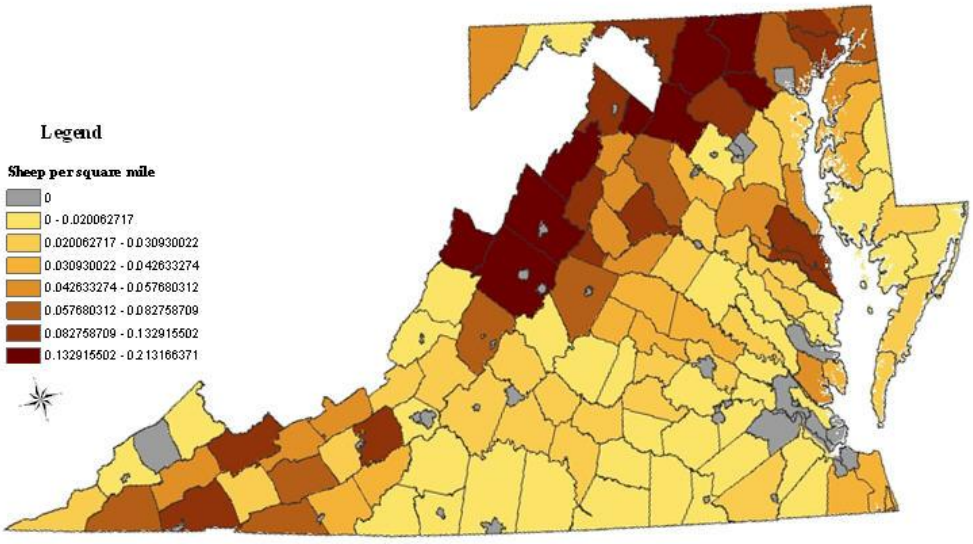

Figure 3.4. Sheep Density in Virginia. The map shows the density of sheep in Virginia as the number of sheep per square mile by county. The data is categorized and displayed in 8 quantiles. Density data for Maryland and the District of Columbia is also shown.

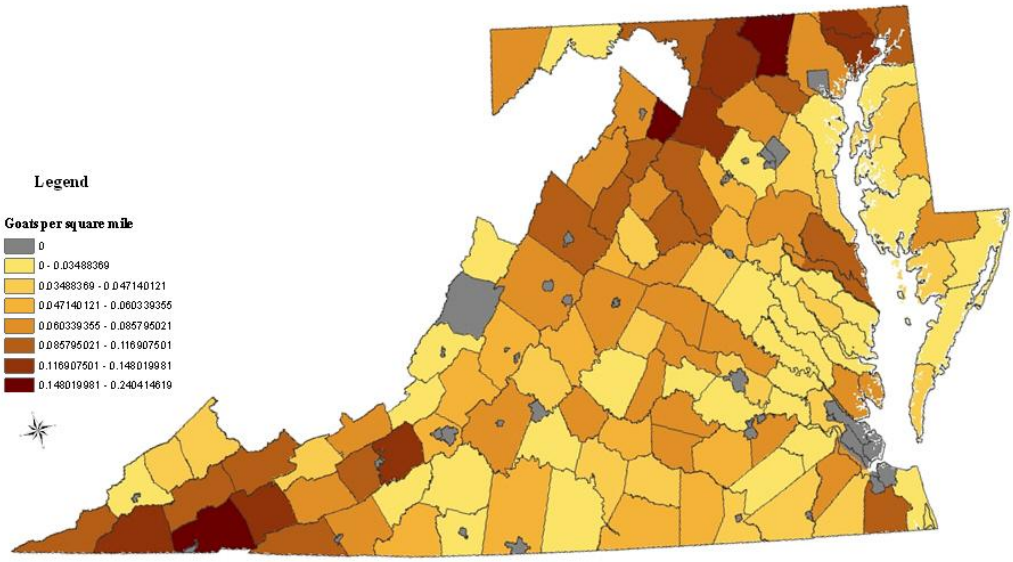

Figure 3.5. Goat Density in Virginia. The map shows the density of goats in Virginia as the number of goats per square mile by county. The data is categorized and displayed in 8 quantiles. Density data for Maryland and the District of Columbia is also shown.

Wildlife Data

Deer population abundance estimates for Virginia for the period of 2000-2006 were obtained from the Virginia Department of Game and Inland Fisheries. The deer abundance estimates are indices rather than densities. They are based on county harvest levels for the state of Virginia and calculated as the number of deer harvested per square mile of public deer habitat (forest, scrub, grass, pasture, crop and woody wetlands), approximately 92% of Virginia (VDGIF 2009). Deer harvest data is not available for all counties in Virginia. For those counties with no data (Figure 3.6), deer abundance was recorded in ESRI ArcGIS Desktop 9.2 (ESRI Inc., Redlands, CA) as 0.00 instead of "no data" so that RVF risk estimates could be calculated for these areas based on vector and other host data.

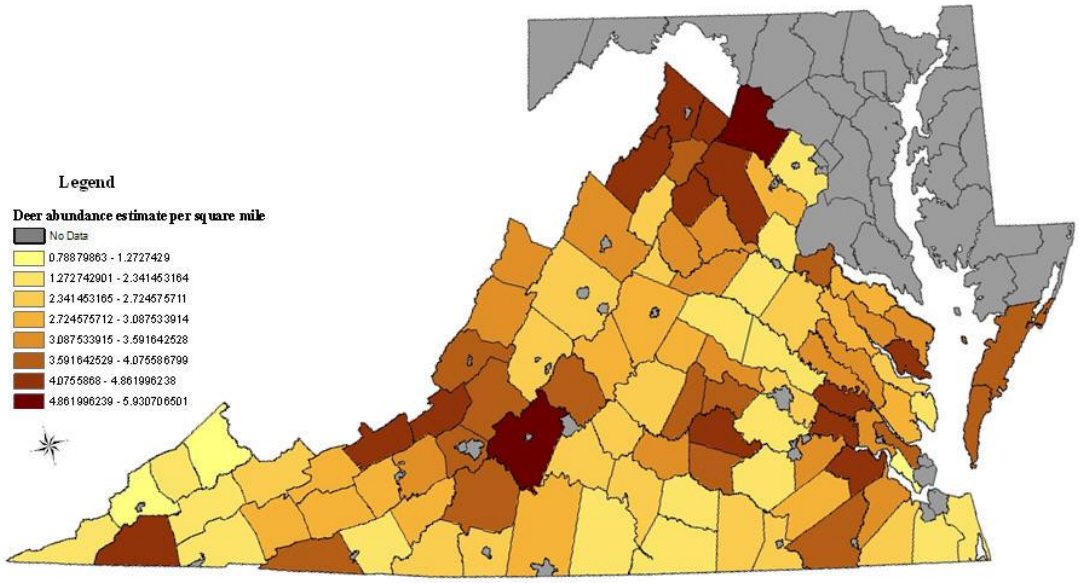

Figure 3.6. 2006 Deer Abundance Estimates for Virginia. The map shows estimates of deer population abundance in Virginia as the number of deer harvested per square mile of public deer habitat by county. The data is categorized and displayed in 9 quantiles.

3.2.3 ENVIRONMENTAL-CLIMATIC DATA SETS

Selection of the environmental-climatic geographic data sets ("coverages") used in this study was based on factors and constraints identified in the review of RVF, mosquito, remote sensing, and ecological niche related literature. That is, the data selected were determined to be suitable for inclusion in a GIS and previously reported to have effects on mosquito biology and to affect environmental suitability for vectors.

The environmental-climatic data sets selected for this study include elevation, land-cover, NDVI, temperature, precipitation, and bioclimatic data. These data were used to evaluate the relationship in Virginia between mosquito abundance and environmental-

climatic variables, to predict mosquito distribution in un-sampled areas of Virginia, and to construct a Virginia RVF risk map.

Elevation

High-resolution (1 km) digital geographic and topographic data obtained from the Shuttle Radar Topography Mission (SRTM) that flew onboard the Space Shuttle Endeavour during an 11-day mission in February of 2000 was downloaded from Worldclim (http://www.worldclim.org). SRTM is an international project spearheaded by the National Geospatial-Intelligence Agency (NGA) and National Aeronautics and Space Administration (NASA) (SRTM 2009). A map of elevation in Virginia is shown in Figure 3.7.

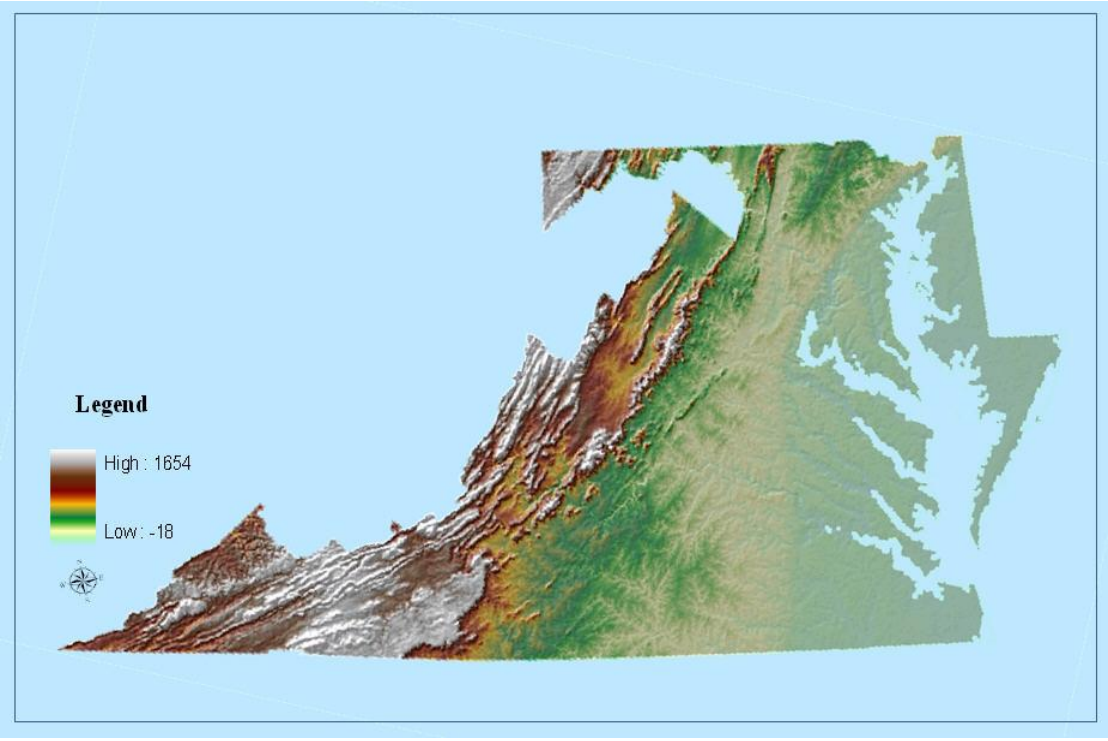

Figure 3.7. Virginia SRTM elevation map.

Land-Cover

Global land-cover data at 1 km resolution were obtained from Boston University's Department of Geography Land Cover and Land Cover Dynamics web site (http://www-modis.bu.edu/landcover/). These data layers are re-projected mosaic exports from MODIS (or Moderate Resolution Imaging Spectroradiometer), a key instrument aboard NASA's Terra satellite. Terra's orbit around the Earth is timed so that it passes from north to south across the equator in the morning. Terra MODIS views the entire Earth's surface every 1 to 2 days, acquiring data in 36 spectral bands, or groups of wavelengths (MODIS 2009). The Boston University product used here (NASA TERRA/MODIS HDF-EOS MOD12Q1 V004) was made from MODIS data from the period 1 Jan 2001 to 31 Dec 2001 and describes the geographic distributions of 17 classes of land cover based on the International Geosphere-Biosphere (IGBP) land cover class scheme (Table 3.3, Figure 3.8).

Table 3.3. Land-cover legend. The geographic distributions of the 17 classes of land-cover are based on the International Geosphere-Biosphere (IGBP) land cover class scheme.

Code	***Category***	***Description***
0	Water	Oceans, seas, lakes, reservoirs, and rivers. Can be either fresh or salt water bodies
1	Evergreen Needleleaf Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 2 meters. Almost all trees remain green all year. Canopy is never without green foliage.
2	Evergreen Broadleaf Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 2 meters. Almost all trees remain green all year. Canopy is never without green foliage.
3	Deciduous Needleleaf Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 2 meters. Consists of seasonal needleleaf tree communities with an annual cycle of leaf-on and leaf-off periods.
4	Deciduous Broadleaf Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 2 meters. Consists of seasonal broadleaf tree communities with an annual cycle of leaf-on and leaf-off periods.
5	Mixed Forests	Lands dominated by trees with a percent canopy cover >60% and height exceeding 2 meters. Consists of tree communities with interspersed mixtures or mosaics of the other four forest cover types. None of the forest types exceeds 60% of landscape.
6	Closed Shrublands	Lands with woody vegetation less than 2 meters tall and with shrub canopy cover is >60%. The shrub foliage can be either evergreen or deciduous.
7	Open Shrublands	Lands with woody vegetation less than 2 meters tall and with shrub canopy cover is between 10-60%. The shrub foliage can be either evergreen or deciduous.
8	Woody Savannas	Lands with herbaceous and other understory systems and with forest canopy cover between 30-60%.The forest cover height exceeds 2 meters.
9	Savannas	Lands with herbaceous and other understory systems and with forest canopy cover between 10-30%.The forest cover height exceeds 2 meters.
10	Grasslands	Lands with herbaceous types of cover. Tree and shrub cover is less than 10%.
11	Permanent Wetlands	Lands with a permanent mixture of water and herbaceous or woody vegetation that cover extensive areas. The vegetation can be present in either salt, brackish, or fresh water.
12	Croplands	Lands covered with temporary crops followed by harvest and a bare soil period (e.g., single and multiple cropping systems. Note that perennial woody crops will be classified as the appropriate forest or shrub land cover type.
13	Urban and Built-Up	Land covered by buildings and other man-made structures. Note that this class will not be mapped from the AVHRR imagery but will be developed from the populated places layer that is part of the Digital Chart of the World.
14	Cropland/Natural Vegetation Mosaic	Lands with a mosaic of croplands, forest, shrublands, and grasslands in which no one component comprises more than 60% of the landscape.
15	Snow and Ice	Lands under snow and/or ice cover throughout the year.
16	Barren or Sparsely Vegetated	Lands exposed soil, sand, rocks, or snow and never has more than 10% vegetated cover during any time of the year.
17	Unclassified	

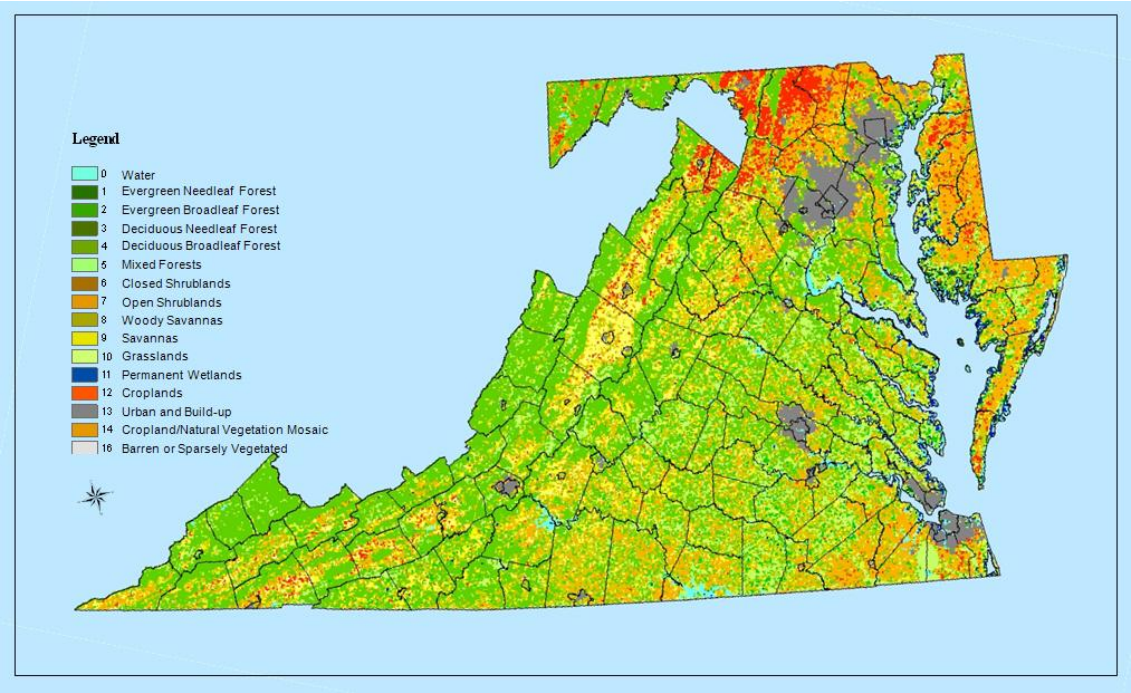

Figure 3.8. Virginia Land-cover

Normalized Difference Vegetation Index

The Normalized Difference Vegetation Index (NDVI) is derived from environmental data collected by the Advanced Very High Resolution Radiometer (AVHRR) sensor on board the National Oceanic and Atmospheric Administration (NOAA) polar-orbiting meteorological satellite. The AVHRR records 5 bands of spectral data for the entire Earth daily. Data recorded from these spectral bands are available at a resolution of 8km x 8km as daily and composite products from 1981 to the present (Estrada-Pena 1998, NASA EO 2008). The 8 km AVHRR NDVI product was selected because 8 km resolution data can blur some landscape heterogeneity present in urban regions of the study area.

Monthly maximum value composite products were obtained for the period of 1981 to 2006. Compositing results in 30-day maximum values which are representative of data collected at near-nadir viewing angles under cloud-free, clear-atmosphere conditions (Tarpley 1984). The raw NDVI values for June 2006 in Virginia are shown in Figure 3.9.

Anomaly NDVI data obtained from Britch *et al.* 2008 and used in the study was calculated as the difference between observed and 25 year mean NDVI for each month in 25 years (1981-2006). That is, ΔNDVI= NDVI - mean NDVI; where ΔNDVI are the respective monthly anomalies, NDVI are the monthly values and mean NDVI are the long-term monthly means, respectively. Anomaly NDVI values provide a measure of positive or negative deviation from the 25-year mean value for each month. Negative values relative to the anomaly NDVI zero line indicate lower-than-average greenness (dry periods) and positive numbers higher-than-average greenness (wet periods) (Britch *et al.* 2008).

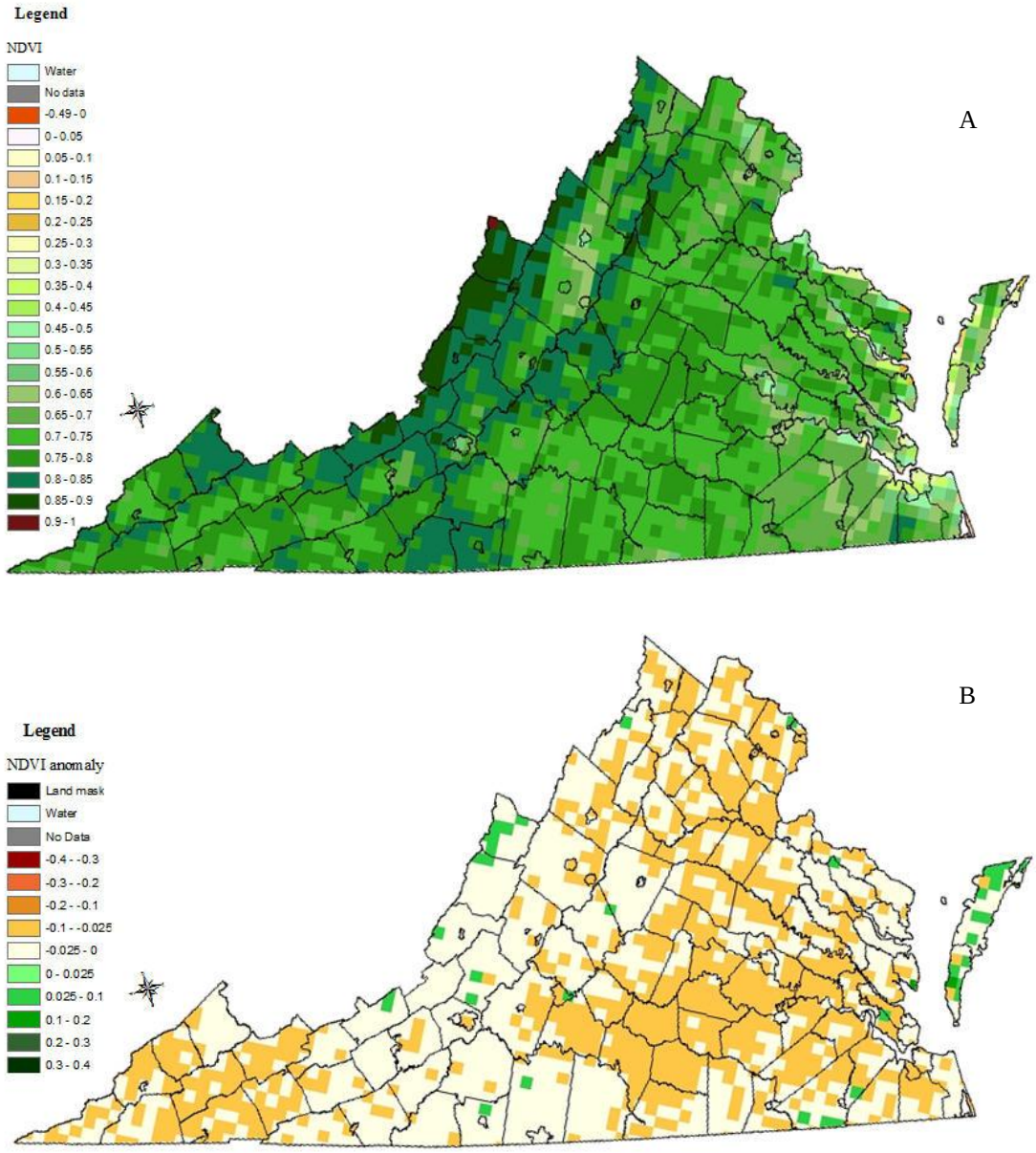

Figure 3.9. June 2006 Normalized Difference Vegetation Index (NDVI) raw and anomaly values in Virginia. Shown in Figure A, values of NDVI for vegetated land generally range from about 0.1 to 0.7, with values greater than 0.5 indicating dense vegetation. Shown in Figure B, the negative values relative indicate lower-than-average greenness (dry periods) and positive numbers higher-than-average greenness (wet periods).

Climate Data

Climate layers generated through interpolation of average monthly climate data for 1950-2000 from weather stations on a 30 arc-second (1 km) resolution grid were obtained from the Worldclim database, version 1.4 (Hijmans *et al.* 2005, http://www.worldclim.org). Variables obtained include average monthly precipitation, average monthly minimum and maximum temperature, and 19 derived bioclimatic variables (Table 3.4). Bioclimatic variables are derived from the monthly temperature and rainfall values in order to generate more biologically meaningful variables often used in ecological niche modeling. The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation), seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters) (Hijmans *et al.* 2005).

Table 3.4. Worldclim interpolated climate layers.

Name	***Description***
tmin	Average monthly minimum temperature (*10), °C
tmax	Average monthly maximum temperature (*10), °C
prec	Average monthly precipitation, mm
bio1	Annual mean temperature, °C
bio2	Mean diurnal range (Mean of monthly (max temp - min temp)), °C
bio3	Isothermality ((bio2 / bio7)*100), °C
bio4	Temperature seasonality (standard deviation *100), °C
bio5	Max temperature of warmest month, °C
bio6	Min temperature of coldest month, °C
bio7	Temperature annual range (bio5 – bio6), °C
bio8	Mean temperature of wettest quarter, °C
bio9	Mean temperature of driest quarter, °C
bio10	Mean temperature of warmest quarter, °C
bio11	Mean temperature of coldest quarter, °C
bio12	Annual precipitation, mm
bio13	Precipitation of wettest month, mm
bio14	Precipitation of driest month, mm
bio15	Precipitation seasonality (coefficient of variation), mm
bio16	Precipitation of wettest quarter, mm
bio17	Precipitation of driest quarter, mm
bio18	Precipitation of warmest quarter, mm
bio19	Precipitation of coldest quarter, mm

Climate layers were also obtained from the PRISM Climate Group (http://www.prism.oregonstate.edu). The 30 arc-second (1 km) resolution data sets available

on this web site were created using the PRISM (Parameter-elevation Regressions on Independent Slopes Model) climate mapping system. PRISM is a unique knowledge-based system that uses point measurements of precipitation, temperature, and other climatic factors to produce continuous, digital grid estimates of monthly, yearly, and event-based climatic parameters (PRISM 2007). Variables obtained include monthly mean precipitation and monthly minimum and maximum temperature for the months in 2005-2006 (Table 3.5).

Table 3.5. PRISM climate layers.

Name	***Description***
ppt	Monthly mean precipitation (mm*100)
tmin	Monthly minimum temperature (°C * 100)
tmax	Month maximum temperature (°C * 100)

3.2.4 ADMINISTRATIVE BOUNDARY DATA SETS

Administrative boundary data sets (Table 3.1) obtained from the ESRI Data & Maps CD included with ArcGIS Desktop 9.2 (ESRI Inc., Redlands, CA) were used to clip other data sets to the geographical extent used in this study and as geographical background reference for the Virginia RVF risk map.

3.2.5 DATA PROCESSING

All data sets were imported into the ESRI GIS software ArcGIS Desktop 9.2 (ESRI Inc., Redlands, CA) as either raster or shape-file formats. The data sets were re-projected to Albers, datum WGS 1984.

Data sets were clipped to an area extending from -74.58 to -83.64 E degrees of longitude and 35.31 to 40.87 N degrees of latitude, corresponding to the geographical limits of Virginia. For regional analyses, data sets were also clipped to the geographical limits of Northern Virginia, Hampton Roads, and Richmond-Petersburg areas of the state.

The original data sets were at a resolution of 1 km or courser. All raster data sets were converted to GRID format at 1 km resolution using the Raster Calculator tool in the ArcGIS Spatial Analyst extension. All data sets were exported as ASCII files for use in the ecological niche modeling software MaxEnt, version 3.2.1 (Phillips *et al.* 2004, Phillips *et al.* 2006).

3.3 IDENTIFICATION OF POTENTIAL RVF COMPETENT VECTORS IN VIRGINIA

Laboratory studies on RVF vector competency for the U.S. were reviewed to identify potential RVF competent mosquito species in Virginia (Gargan *et al.* 1988, Turell *et al.* 1988, Turell *et al.* 2005). The assumption was made that if laboratory RVF competent vectors are found in Virginia, then Virginia's climatic and environmental conditions are favorable to support these vectors and, consequently, RVF virus if it is introduced in this state. Also, the assumption was made that if a native U.S. vector can transmit RVF virus in the laboratory, then it could also transmit the virus in the wild.

The ability to become infected and transmit the virus in the laboratory does not necessarily mean that the species will play a significant role in transmitting the virus in nature (Chevalier *et al.* 2004a, Turell *et al.* 2005). Consequently, factors that affect how important a particular species will be in transmitting RVF virus (e.g., population density, host-feeding preference, time of day of feeding, and behavior) (Turell *et al.* 2005) were also considered in selecting mosquito species to be included in this study.

Finally, a literature review of WNV studies (Dohm and Turell 2001, Turell *et al.* 2001, Hayes *et al.* 2005, Allen and Wong 2006, Duik-Wasser *et al.* 2006, Brown *et al.* 2008, Turell *et al.* 2008) examining distribution of mosquitoes potentially capable of transmitting both WNV and RVF virus was also accomplished to help narrow down the list of species to be included in this study.

3.4 Mosquito Surveillance Database Development and Statistical Analysis

Mosquito surveillance records for 2000-2006 were gathered, standardized and compiled in a Microsoft Office Access 2003 (Microsoft Corporation) database. In the counties[2] with available mosquito surveillance data, mosquito traps were placed in consistent locations and produced data for at least part of the time period of the study. However, mosquito surveillance methods were not uniform across surveillance districts; mosquito population samples were collected from an array of trap types (e.g., light traps, gravid traps) which may sample different elements of mosquito communities. In addition, districts potentially trapped over different time spans in a month. To control the variance and

[2] Virginia is divided into independent cities and counties, which function in the same manner. In this study, "county" will refer to both counties and cities in Virginia.

normalize differences among surveillance methods, a monthly index of the mean number of female mosquitoes caught per trap per night was calculated, log-transformed and multiplied by 10 prior to analysis[3] (Sadanandane *et al.* 2004).

The mosquito surveillance database was imported to ArcGIS Desktop 9.2 (ESRI Inc., Redlands, CA), an integrated collection of GIS software. In the ArcMap application, traps were geocoded[4], plotted, and linked via attribute tables to the mosquito surveillance data. Mosquito counts for which there was uncertainty about the location of the sampling were excluded. Spatial maps were created to show trap site locations and mosquito density at the trap sites for each of the 9 mosquito species considered to be potential competent RVF vectors.

The majority of surveillance data evaluated in this study were for the period of 2005-2006 and from the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions of the state, however, data from 2000-2006 from all regions of the state was compiled to represent the full range of ecological conditions over the longest period of time possible. Using Microsoft Access, ArcMap, and SigmaPlot 11.0 (Systat Software, Inc.), the mosquito surveillance data at the state, regional, and county level was explored and analyzed, focusing on the 9 mosquito species identified as potential RVF competent mosquito species in Virginia. An abundance index was calculated to measure number of mosquitoes collected relative to the number of trap nights.

[3] Referred to throughout as "monthly mean mosquitoes" or "mosquito counts" or "average females."
[4] The Virginia Department of Transportation (VDOT) (http://gis.virginiadot.org/) assisted with geo-coding some trap sites.

3.5 Evaluation Of Mosquito Density – Environmental-Climatic Relationships

The Normalized Difference Vegetation Index (NDVI) is a proven biologically realistic index of environmental factors influencing mosquito populations. Animal life depends on vegetation, so tracking spatial-temporal variation in vegetation similarly evaluates animal populations (Hielkema *et al.* 1986). The goal in these regional analyses was to evaluate the relationship or association between NDVI and Virginia mosquito density. Since a species is sampled at multiple locations and NDVI raw and anomaly values vary among locations, mosquito density-NDVI relationships were examined spatially within the 2005-2006 time period for each region as well as temporally by region on a monthly time scale. In the analysis of these relationships, the null expectation or hypothesis is that the mosquito density-NDVI relationships across space and across time are the same, and forms the basis for using NDVI to develop a predictive model for un-sampled areas in Virginia.

Temperature and precipitation (in addition to NDVI) have previously been reported to affect mosquito biology (Alto and Juliano 2001, Turell *et al.* 2005). Environmental-climatic variables (temperature, precipitation, and NDVI) were evaluated to determine which variable is a better predictor of mosquito distribution and abundance.

Data preparation

Data was not available for all counties for 2000-2006. The majority of the reliable data was for 2005-2006 for the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions. Therefore, only mosquito surveillance data and predictor data (NDVI, elevation, temperature, precipitation, and temperature-rainfall derived values) for 2005-2006

for three regions of Virginia – Northern Virginia, Richmond-Petersburg, and Hampton Roads – were used in temporal analyses.

The mosquito surveillance data supporting analyses of the **Northern Virginia** region were from seven counties: Fairfax County, Fairfax City, Loudoun County, Alexandria City (2006 only), Arlington County, Prince William County, and Falls Church City. Four records had inaccurate geographic coordinates that placed the mosquito counts outside of the Northern Virginia region; these records were discarded. A total of 2,581 records[5] for 2005 and 3,274 records for 2006 were included in analyses. The Manassas City and Manassas Park City jurisdictions in the Northern Virginia region were included in the display of results.

The mosquito surveillance data supporting analyses of the **Richmond-Petersburg** region were from five counties: Henrico County, Richmond City, Chesterfield County, Petersburg City, Goochland County. A total of 1,411 records for 2005 and 1,405 records for 2006 were included in analyses. Surrounding jurisdictions (Hopewell City, Charles City County, Dinwiddie County, Hanover County, New Kent County, Powhatan County, Prince George County, Colonial Heights City) in the Richmond-Petersburg region were included in the display of results.

The mosquito surveillance data supporting analyses of the **Hampton Roads** region were from seven counties: Newport News City, Hampton City, Virginia Beach City, Suffolk City, Chesapeake City, Portsmouth City, and Norfolk City. A total of 4,008 records for 2005 and 5,321 records for 2006 were included in analyses. Surrounding jurisdictions (Isle of Wright County, James City County, York County, Surry County, Gloucester County,

[5] Each record is the mean monthly mosquito count of a potential RVF competent vector species collected at given trap site.

Mathews County, Southampton County, Poquoson City, and Williamsburg City) in the Hampton Roads region were included in the display of results.

Regional analyses were accomplished for each species (*Ae. albopictus*, *Ae. canadensis*, *Ae. taeniorhynchus*, *Ae. triseriatus*, *Ae. vexans*, *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*) and for a "competent-vector" group that included data for all 9 species identified as potential RVF competent vectors in Virginia. *Ae. sollicitans* and *Ae. taeniorhynchus* species were not included in individual species analyses because the sample size for these species was too small for statistically significant analyses.

The Sample tool in the ArcGIS Spatial Analyst extension was used to extract predictor variable raster values for each pixel of the study area and for each pixel that corresponded to mosquito surveillance points (trap sites). Extracted data were imported to SigmaPlot 11.0 for statistical analysis. The mean and range were calculated for each variable.

State and regional graphical analyses

For each region, monthly mosquito indices (for each species and for the competent-vector group) were plotted against monthly and long-term mean values for NDVI anomaly, raw NDVI, mean precipitation, and mean maximum and minimum temperature. Graphs were examined for spatial and temporal trends. In particular, these graphs were used as an initial assessment of whether relationships exist in Virginia between mosquito populations and greenness or greenness anomalies, indicated by NDVI values.

Correlation analyses

The Pearson's Product-Moment Correlation (PPMC) Coefficient in SigmaPlot 11.0 was used to measure the associations among predictor variables and the associations between predictor variables and mosquito abundance in the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions. The test was also used to evaluate which variables would be better predictors for inclusion in a predictive model for un-sampled areas in Virginia.

Only predictor data for months in which traps were placed (April-October) were used in the statistical analyses to examine the relationship between mosquito counts and predictor variables. The independent variables were monthly mean minimum and maximum temperature, monthly mean precipitation, and monthly mean maximum NDVI. The dependent variable was the number of mosquitoes per trap per month.

3.6 Prediction of Competent Vector Distribution

The overall objective of this study was to produce maps showing the risk of RVF virus establishment and transmission to humans in Virginia. Before the risk maps could be created, it was necessary to produce a distribution map for the RVF competent vectors in Virginia. To generate predictions of mosquito distributions over wide areas, it is rarely practical to carry out new mosquito surveys, as these would have to cover the whole region described by the required risk map. Instead, this type of study tends to rely on species distribution or ecological niche modeling methods. Ecological niche modeling methods use species presence data to predict the probability of occurrence or estimate the environmental suitability for the species as a function of a set of selected environmental variables (Peterson 2006). This technique is increasingly being used to model distribution of diseases such as

malaria and leishmaniasis, and vectors such as *Anopheles gambiae* (Peterson and Shaw 2003, Levine *et al.* 2004, Peterson *et al.* 2004, Peterson *et al.* 2005).

MaxEnt Program for Ecological Niche Modeling

This study utilizes a maximum entropy approach to develop an ecological niche model of the distribution of RVF competent vectors across the state of Virginia.[6] MaxEnt version 3.2.1 (Phillips *et al.* 2004, Phillips *et al.* 2006), the application used here, is easy to use, batchable, and has produced useful predictions of species distributions, likelihood of establishment, and alterations of ranges caused by climate change (Phillips *et al.* 2004, Hernandez *et al.* 2006). Having presence-only data for this study, MaxEnt was a reasonable method to select because it does not require an explicit quantification of absence to formulate a predicted distribution model (Phillips *et al.* 2004). In addition, Elith *et al.* 2006 found that MaxEnt was one of the strongest performers in a large model comparison study. Also noteworthy, Hernandez *et al.* (2006) evaluated four species distribution modeling methods and concluded that MaxEnt was the most capable in producing useful results with stable prediction accuracy even when sample sizes were small.

MaxEnt produces predictions from incomplete information by estimating the most uniform distribution (maximum entropy) of occurrence points across the study area. MaxEnt is designed to estimate a target probability distribution by finding the probability distribution

[6] Initially co-kriging, a common geostatistical technique that provides a means of using predictor variables to interpolate values for unrecorded locations and calculate a measure of variance around estimated values, was considered for these analyses. However, after an initial attempt to interpolate mosquito abundance and distribution using environmental-climatic variables it was evident that co-kriging could not adequately estimate mosquito values because the surveillance data was clustered in three region (Northern Virginia, Richmond-Petersburg, and Hampton Roads).

of maximum entropy (i.e., that is most spread out, or closest to uniform), subject to a set of constraints that represent the incomplete information about the target distribution. The information available about the target distribution is a set of real-valued variables, called "features", and the constraints are that the expected value of each feature should match its empirical average (average value for a set of sample points taken from the target distribution) (Phillips *et al.* 2006). When MaxEnt is applied to presence-only species distribution modeling, each occurrence locality or sample point is a latitude–longitude pair denoting a site where the species has been observed (in this case, mosquito trap sites). The geographic region of interest (e.g., Virginia) is the space on which the distribution is defined. The features are environmental and climatic variables (e.g., NDVI, temperature, precipitation, elevation, land-cover, and bioclimatic data) (Phillips *et al.* 2004).

MaxEnt establishes the relationship between the predictor variables and the presence of the species being modeled. MaxEnt predictions for each analysis cell or pixel are ''cumulative values'', represented as a percentage, the probability value for the current analysis cell and all other cells with equal or lower probability values. The cell with a value of 100 is the most suitable, while cells close to 0 are the least suitable within the study area (Phillips *et al.* 2004, Hernandez *et al.* 2006).

MaxEnt Model Building and Evaluation

A RVF competent vector distribution map was made by relating observed mosquito presence data to environmental and climatic variables. The following environmental and climatic variables were included in analyses: Worldclim average monthly temperature (maximum and minimum), Worldclim average monthly precipitation, 19 Worldclim

bioclimatic (Table 3.4), land-cover, SRTM elevation, and monthly maximum composite NDVI. Prior to importing the data to MaxEnt, the Map Algebra tool in the ArcGIS Spatial Analyst extension was used to create minimum, mean, and maximum NDVI coverages using monthly 2000-2006 NDVI data. The Map Algebra tool was also used to create minimum, mean, and maximum precipitation coverages using the Worldclim average monthly mean precipitation data as well as minimum and maximum temperature coverages using the Worldclim average monthly minimum and maximum temperature data. The mosquito presence data used in these analyses was a "competent-vector" group that included 2000-2006 data for all 9 species identified in this study as potential RVF competent vectors in Virginia. The MaxEnt model output was set to logistic, which returns an estimated probability of suitable habitat for species presence for a given location between the values of 0 (no probability of species presence) and 1 (species is certain to be present). The MaxEnt cumulative model output was also tried; this output returns an estimated probability of suitable habitat for species presence for a given location between the values of 0 and 100. All other parameters were set to the default settings.

The MaxEnt program was set to calculate jackknife tests of variable importance in order to get estimates of which variables contribute most in model development. The jackknife procedure produces three different types of models: (1) models created by excluding one variable at a time while all other variables are included, (2) models created by including only one variable at a time, and (3) models created by including all variables (Phillips *et al.* 2006, Phillips and Dudik 2008). Variables that are most important to model development are those that decrease the training gain when removed from the model and show gain when the model is developed with only one variable.

When test data are available, MaxEnt automatically calculates the statistical significance of the prediction using a binomial test of omission. Therefore, to be able to evaluate model performance (the accuracy of predictions), MaxEnt was allowed to randomly partition the mosquito data into training and test data sets. Phillips *et al.* 2004 found that about 50-100 training samples is sufficient to obtain a prediction that is close to optimal. For this model, the number of samples was relatively large (1231) even after duplicate presence records were removed by the MaxEnt program prior to model development. Consequently, fifty percent of the mosquito occurrence data points (616) were randomly selected as training points and were used in model building. The remaining 50% (615) of the records were test points, used in model validation. There were a total of 10592 points (background and presence cells in the extent) used to determine the RVF competent vector distribution in Virginia.

The accuracy of model predictions was evaluated using both threshold-dependent and threshold independent methods. The threshold-dependent measure used here is the minimum training presence in which the probabilities are converted to binomial values with 0 being absent and 1 being present (Phillips *et al.* 2006, Phillips and Dudik 2008). Using this method, all pixels with a probability of presence equal to or greater than that of the training point with the lowest probability of presence are classified as present, and all pixels with a lower probability of presence are classified as absent. A one-tailed binomial test is then performed with the null hypothesis being that the model does not predict the test points better than random (Phillips *et al.* 2006).

MaxEnt uses threshold-independent measures to calculate the area under the curve (AUC) of a receiver operating characteristic (ROC) plot of sensitivity against specificity.

Sensitivity is defined as the proportion of true positives correctly predicted (e.g., the percentage of locations where mosquito presence is correctly predicted), whereas specificity is the proportion of true negatives correctly predicted (e.g., the percentage of locations where mosquito absence is correctly predicted) (Fielding and Bell 1997, Phillips *et al.* 2006, Phillips and Dudik 2008). A model with perfect discrimination between presence and absence will have an AUC of 1.0. As a general rule, an AUC between 0.5 and 0.7 indicates a poor discriminative capacity; 0.7-0.9 indicates a reasonable capacity; and > 0.9 indicates a very good capacity (Brooker *et al.* 2001, Phillips *et al.* 2006, Phillips and Dudik 2008). The distribution map resulting from the "best model" (highest AUC) represents the best approximation of the species' realized niche, as a function of the given environmental variables of the study area. This map was subsequently exported to ArcGIS 9.2 for use in the development of a Virginia RVF risk map.

3.7 Virginia Rift Valley fever Risk Map Development

A weighted suitability model was developed using the ArcGIS Spatial Analyst extension to create risk maps that visually represent the variation in risk of RVF virus establishment across Virginia and risk of RVF transmission to humans in Virginia. RVF virus establishment and transmission to humans is likely to occur where competent mosquito vectors are present near host populations. Therefore, the model included the MaxEnt Mosquito Habitat Suitability model as the layer representing competent vector mosquito distribution in Virginia and three layers representing competent host (domestic animal, wildlife, and human) density in Virginia. The domestic animal layer, created using the Map

Algebra tool in the ArcGIS Spatial Analyst extension, included county level combined numbers of cattle, sheep and goat per square mile (Figure 3.10).

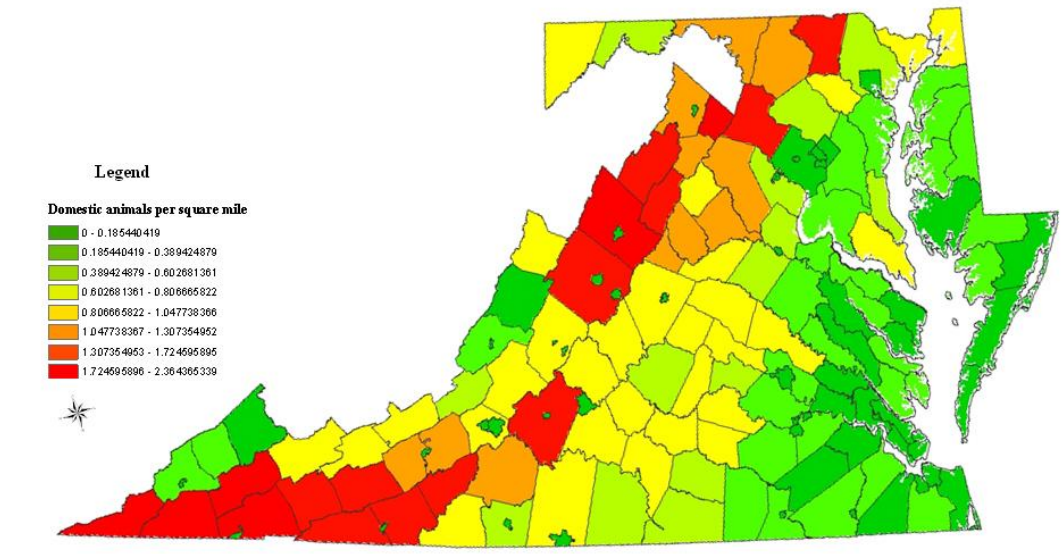

Figure 3.10. Domestic Animal Density in Virginia. The map shows the density of domestic animals (cattle, sheep, and goats) in Virginia as the number of animals per square mile by county. The data is categorized and displayed in 8 quantiles. Density data for Maryland and the District of Columbia is also included.

In the weighted suitability model, each data layer was first reclassified on a relative scale (0-9 scale) in order to allow comparison between the variables. Each reclassified layer was then multiplied by a weight that indicates its estimated relative importance in RVF virus establishment or RVF virus transmission to humans. The weights summed to 1.0 so that the weight of a layer was equivalent to the percentage importance of each category in the final model. This process was somewhat subjective, based on a review of RVF literature related to

outbreaks in endemic regions and on knowledge of vector and host population densities and roles in zoonotic diseases in Virginia. The results of the weighted suitability model were maps indicating the probability of risk of RVF virus establishment and risk of RVF virus transmission, based on the overlap of host and vector layers. Values for each cell on the maps were displayed by color gradation to show low to high risk by geographic. Steps in the development of the risk maps were included in the ArcGIS ModelBuilder application so that the affects of changes to the model and weights of variables could be evaluated.

CHAPTER 4. RESULTS

4.1 POTENTIAL RVF COMPETENT VECTORS IN VIRGINIA

Of the approximately 57 different species of mosquitoes found in Virginia, nine mosquito species were selected for this study based on the following:

A review of studies on RVF outbreaks. In Africa more than 30 different species of mosquitoes in the genera *Aedes*, *Anopheles*, *Culex*, *Eretmapodites*, and *Mansonia* have been found infected with RVF virus during outbreaks (Meegan and Bailey 1988, Traore-Lamizana *et al.* 2001).

A review of RVF vector competency laboratory studies. Under laboratory conditions RVF virus has been biologically transmitted by numerous mosquitoes including members of the genera *Aedes*, *Anopheles*, *Culex*, and *Eretmapodites* (Meegan and Bailey 1988, House *et al.* 1992). For instance, Gargan *et al.* (1988) selected and evaluated North American mosquito species as potential vectors of RVF virus. For most of the species, about half of the mosquitoes with a disseminated infection transmitted an infectious dose of virus to hamsters. Gargan *et al.* 1988 concluded that if RVF virus was introduced into North America, several mosquito species known to commonly feed on large mammals and humans would be capable of transmitting the virus. The vector potential ranged from very good for *Ae. canadensis*, *Ae.*

taeniorhynchus, and *Cx. tarsalis* to very poor for *An. bradleyi- crucians*[1]. Intermediate levels of vector potential were recorded for the other *Culex* species (*salinarius* and *territans*) and *Aedes* species (*cantator, sollicitans* and *triseriatus*) (Gargan *et al.* 1988). Dissemination from the midgut appears to be the primary determinant of vector competence. RVF vector competency studies and the species found to be competent vectors are listed in Table 4.1.

An initial analysis of Virginia mosquito surveillance data to estimate species presence and abundance followed by a study of the bioecology of the species with distributions in Virginia. The ability to become infected and transmit the virus in the laboratory does not necessarily mean that the species will play a significant role in transmitting the virus in nature (Chevalier *et al.* 2004a, Turell *et al.* 2005). Consequently, an initial analysis of Virginia mosquito surveillance data was performed and the bioecology of mosquitoes with distributions in Virginia was examined to evaluate factors that affect how important a particular species will be in transmitting RVF virus (e.g., population density, host-feeding preference, feeding time of day, and behavior) (Traore-Lamizana *et al.* 2001, Turell *et al.* 2005).

Assumptions. The following two assumptions were made:

[1] At the time of the Gargan *et al.* 1988 study, the species *canadensis, cantator, sollicitans, taeniorhynchus,* and *triseriatus* were classified in the genus *Aedes*, subgenus Ochlerotatus. Reinert 2000 and Reinert *et al.* 2004 elevated the subgenus Ochlerotatus to a genus based on microscopic differences in the male genitalia between the Ochlerotatus subgenus and other *Aedes* subgenera. However, in 2005 the American Journal of Tropical Medicine and Hygiene (Weaver 2005), the Journal of Medical Entomology, and the Entomological Society of America rejected the elevation of Ochlerotatus subgenera to generic rank and encouraged authors to return to the former classification method as noted in "Journal Policy on Names of Aedine Mosquito Genera and Subgenera", J. Med. Entomol 42(5):511 (2005). The guidelines established by these journals are followed in this study.

1. If a native U.S. vector can transmit RVF virus in the laboratory, then it could transmit the virus in the wild.
2. For a vector-borne disease such as RVF to become established and cause disease, the pathogenic organism must be able to survive, proliferate, and find a way to enter a susceptible host (Wilson 1995). It was assumed that if environmental conditions are favorable to potential U.S. RVF competent vectors then the conditions are or could become favorable for the development, spread and establishment of the RVF virus.

The nine species in Table 4.1 should be considered potentially important vector for RVF virus in Virginia and were therefore selected for this study because of high population densities in areas of the state, detection of RVF virus in the species, laboratory transmission of RVF virus in the species, and/or preference for feeding on mammals.

Table 4.1. Mosquito species selected for analysis. Justification for selection was based on host feeding preference, number of mosquitoes collected in Virginia relative to the number of trap nights, transmission of the virus in RVF outbreaks, and demonstrated competency in laboratory studies.

Mosquitoes Selected for Analysis	***Host Preference***	***Total # of Mosquitoes (2005-2006)***	***# of Trap Nights***	***Average # of Females***	***RVF outbreaks***	***Demonstrated competency in laboratory studies***
Aedes albopictus	Opportunistic	60585	12473	4.86		Biological: Turell *et al.* 1988
Aedes canadensis	Mammals	31693	3687	8.60		Biological: Gargan *et al.* 1988
Aedes sollicitans	Mammals	1197	487	2.46		Biological: Gargan *et al.* 1988
Aedes taeniorhynchus	Mammals	1752	1010	1.73		Biological, mechanical: Gargan *et al.* 1988, Hoch *et al.* 1985
Aedes triseriatus	Mammals	5315	6626	0.80		Gargan *et al.* 1988
Aedes vexans	Mammals	71176	12517	5.69	Senegal 1993, Fontenille *et al.* 1948	
Culex pipiens	Birds, also Mammals	99338	8543	11.63	Egypt 1977 and 1978; Hoogstraal *et al.* 1979, Meegan *et al.* 1980	Biological, mechanical: Hoch *et al.* 1985, Turell *et al.* 1996, Turell *et al.* 2008
Culex pipiens/restuans		95463	3701	25.79		
Culex restuans	Birds	134164	10639	12.61		N/A*
Culex salinarius	Opportunistic	70485	8509	8.28		Biological: Gargan *et al.* 1988

* No RVF vector competency laboratory studies have included *Cx. restuans*. This species was selected due to high numbers of *Cx. restuans* collected in Virginia. In addition, in 2005-2006 a total of 95,463 mosquitoes were collected in Virginia and only identified to the genus level (*Cx.* spp.). Because *Culex* spp. have been found to transmit RVF virus during outbreaks and in laboratory studies, *Cx. restuans* were considered to be potential RVF competent vectors in Virginia.

Bioecology of Species Selected for the Study

Aedes (Ae.) albopictus is a container-breeder, breeding in treeholes as well as man-made containers such as tires and cans (Paulson 2006). Although *Ae. albopictus* feeds on many different hosts (opportunistic), it is an avid man-biter. Unlike many mosquitoes, it is very active during the day. This species is especially prevalent in urban and suburban habitats (Paulson 2006). *Ae. albopictus* is well adapted to anthropogenic breeding sites, such as tires, and is frequently found in urban areas (Hay *et al.* 2005). In a RVF vector competency study of a Houston, TX strain of the species, Turell *et al.* (1988) reported a 15% transmission rate for *Ae. albopictus* with disseminated infection. Although this appears to be a low rate, it is comparable to the rate observed in *Ae. mcintoshi*, one of the RVF virus African vectors. Turell *et al.* (1988) concluded that this species should be considered a potential vector of RVF virus if the virus is introduced into the southern United States.

Ae. canadensis was included in this study based on its local abundance and vector competence (Gargan *et al.* 1988). In addition, *Ae. canadensis* preferentially feeds on a broad range of animals, including large and small mammals. Larvae of the species hatch from overwintering eggs during the early spring most commonly in shallow, leaf-lined pools in wooded areas but can be encountered in deep snow pools, roadside ditches, vernal pools in open fields, along the edges of permanent swamps, and in acid water bogs (Horsfall 1955, Crans 2004).

Ae. sollicitans was included in this study based on its local abundance, vector competence (Gargan *et al.* 1988), and preferential mammalian feeding behavior. *Ae. sollicitans* lay eggs individually on moist substrate around despressions at the upper reaches of grassy salt marshes (Horsfall 1955, Crans 2004). It is a major pest species on the eastern

seaboard of the United States where salt marshes are prevalent, and may be an important vector of both Eastern equine encephalitis (EEE) and Venezuelan equine encephalitis (VEE) (Turell *et al.* 2005).

Ae. taeniorhynchus was included in this study based on its local abundance and vector competence (Gargan *et al.* 1988, Hoch *et al.* 1985). In addition, it is a severe biter of humans and livestock along the eastern seaboard of the United States where it breeds in salt marshes. *Ae. taeniorhynchus* mosquitoes are efficient vectors of VEE (Turell *et al.* 2005).

Ae. triseriatus is a potential bridge vector of West Nile virus (WNV) and is the primary vector of La Crosse encephalitis (LAC) in southwest Virginia. It is common near areas of human habitation where it feeds on a variety of mammals and breeds in man-made containers and treeholes (Paulson 2006). The species is included in this study because it has been found to transmit RVF virus in laboratory studies (Gargan *et al.* 1988).

Ae. vexans is an opportunistic feeder, willing to utilize a wide variety of hosts including humans, sheep, and horses (Traore-Lamizana 1997). It breeds in many different kinds of habitats including temporary and semi-permanent ground pools, floodplains, ditches, and grassy rain pools (Paulson 2006). *Ae. vexans* is one of the most common floodwater mosquito species found anywhere in VA. It has the most cosmopolitan geographic distribution in Virginia, has multiple generations, and is active from April to October (Gaines, pers. comm.). Its inclusion in this study is based on its local abundance, vector competence, aggressive mammalian biting behavior, and infection with RVF in natural outbreaks (Fontenille *et al.* 1998).

Culex (Cx.) pipiens and ***Cx. restuans*** are common in urban, suburban, and rural locations and breed in a wide variety of habitats including catch basins, ground pools,

ditches, animal waste lagoons, and artificial containers (Paulson 2006). These species are largely ornithophilic (Apperson *et al.* 2004, Turell *et al.* 2005), however, *Cx. pipiens* and *Cx. restuans* will feed on mammals, including humans and white-tailed deer, when these hosts are abundant (Jackson and Paulson 2006, Molaei *et al.* 2006, Paulson 2006, Patrician *et al.* 2007).

Cx. pipiens mosquitoes were the principal vector for human-to-human transmission of RVF virus during the 1977-1978 RVF outbreak in Egypt. In a RVF vector competency study of Egyptian mosquito species, *Cx. pipiens* infection, dissemination, and transmission in laboratory studies was similar for specimens collected in Egypt. Virtually all individuals that develop disseminated infection would be expected to transmit the virus by bite (Turell *et al.* 1996).

Although no RVF vector competency laboratory studies have included *Cx. restuans*, its inclusion in this study is based on the fact that other *Culex* species have been found to transmit RVF virus during outbreaks and in laboratory studies. *Cx. restuans* was also included due to local abundance in Virginia.

Cx. salinarius is a more catholic feeder that readily bites mammals (Andreadis *et al.* 2004, Molaei *et al.* 2006, Brown *et al.* 2008). Based on this and results of vector competency studies, *Cx. salinarius* would likely be involved in transmission of RVF virus to humans in the United States. Virtually any freshwater habitat with dying vegetation can support *Cx. salinarius* larvae. The larvae can also develop where brackish conditions are found along salt marshes (Horsfall 1955, Crans 2004).

Table 4.2 summarizes the bioecology of the species selected for this study.

Table 4.2. Bioecology of potential RVF competent vectors in Virginia.*

Mosquito Species	***Typical Habitat***	***Host Preference***	***Other Characteristics***	***Active Time***	***Flight Range***	***Association with other viruses***	***Vector Competence for RVF***
Ae. albopictus	Tree holes: peridomestic	Opportunistic	Especially prevalent in urban/ suburban habitats, desiccation resistant eggs laid above waterline in container habitat, multiple generations each year, overwinters in diapausing egg stage	Crepuscular/day	200 m	EEE, LAC, WNV	<10%[a]
Ae. canadensis	Most common in shallow, leaf-lined pools in wooded areas	Mammals	Desiccation resistant eggs laid in ground depressions, single generation (univoltine) in early spring, but frequently reappear more than once during a single breeding season, overwinters in the egg stage,	Day	2 km	EEE	>40%[a]
Ae. sollicitans	Salt marshes	Mammals	Desiccation resistant eggs laid on a substrate that will be flooded by lunar tides, larvae develop in salt marsh pools, multiple generations each year, overwinters in the egg stage	Crepuscular/night	>25 km	EEE	25-40%[a]
Ae. taeniorhynchus	Salt marshes	Mammals (Severe biter of humans and livestock)	Desiccation resistant eggs laid on a substrate that will be flooded by lunar tides, larvae develop in salt marsh pools, multiple generations each year, overwinters in the egg stage	Day and night	>25 km	EEE	>40%[a]
Ae. triseriatus	Tree holes: peridomestic, utilizes man-made containers such as tires and cans	Mammals	Common near areas of human habitation, desiccation resistant eggs laid above the waterline in a container habitat, multiple generations each year, overwinters in a diapausing egg stage	Day	200 m	LAC, WNV	11-25%[a]

Mosquito Species	*Typical Habitat*	*Host Preference*	*Other Characteristics*	*Active Time*	*Flight Range*	*Association with other viruses***	*Vector Competence for RVF*
Ae. vexans	Variety of habitats including temporary and semi-permanent ground pools, floodplains, ditches, and grassy rain pools	Mammals	Desiccation resistant eggs laid in ground depressions, multiple generations each year (multivoltine), overwinters in the egg stage;	Crepuscular/night	>25 km	EEE, WEE, SLE	
Cx. pipiens	Stagnant water pools; common in urban, suburban, and rural locations. Breed in a wide variety of habitats including catch basins, ground pools, ditches, animal waste lagoons, and artificial containers	Birds, also Mammals (including humans and white-tailed deer, when these hosts are abundant)	Non-desiccation resistant eggs laid directly on water, multiple generations each year (multivoltine), winters as a mated female	Crepuscular/night	2 km	SLE	25-40%[a]
Cx. restuans	Stagnant water pools; common in urban, suburban, and rural locations. Breed in a wide variety of habitats including catch basins, ground pools, ditches, animal waste lagoons, and artificial containers	Birds	Non-desiccation resistant eggs laid directly on water, multiple generations each year (multivoltine), winters as a mated female	Crepuscular/night	2 km	EEE, SLE	[a]
Cx. salinarius	Fresh or foul water pools, greatest abundance in areas adjacent to salt marshes where fresh water from the upland drains onto coastal habitats	Opportunistic	Non-desiccation resistant eggs laid directly on water, multiple generations each year (multivoltine), overwinters as a mated female	Crepuscular/night	10 km	EEE, SLE	<10%[a]

*Partly adapted from Turell *et al.* 2005. They based and generalized distribution and bionomics from information in Carpenter and LaCasse (1955), Darsie and Ward (1981), and Moore *et al.* (1993). Additionally, information came from Horsfall (1955) and Crans (2004).
**Known association with other viruses with a similar transmission cycle: EEE, eastern equine encephalomyelitis virus; LAC, La Crosse encephalitis; SLE; St. Louis encephalitis virus; WEE; western equine encephalomyelitis virus. Adapted from Turell *et al.* 2005, Based on Karabatsos (1985).

4.2 VIRGINIA MOSQUITO SURVEILLANCE STATISTICS

Statistical analyses of RVF competent vector mosquito surveillance data gathered by mosquito control districts in Virginia revealed that a total of 1,168,131 competent vector mosquitoes were collected in 110,772 trap nights from 2,101 different trap locations over the period of 2000-2006.[2] The monthly mean mosquito index[i] was 15.63. There were a total of 29,766 competent vector records[ii] available for analyses in this study. Figure 4.1 is a map showing the location of the mosquito surveillance traps sites in Virginia from which the mosquito population data used in this study was collected.

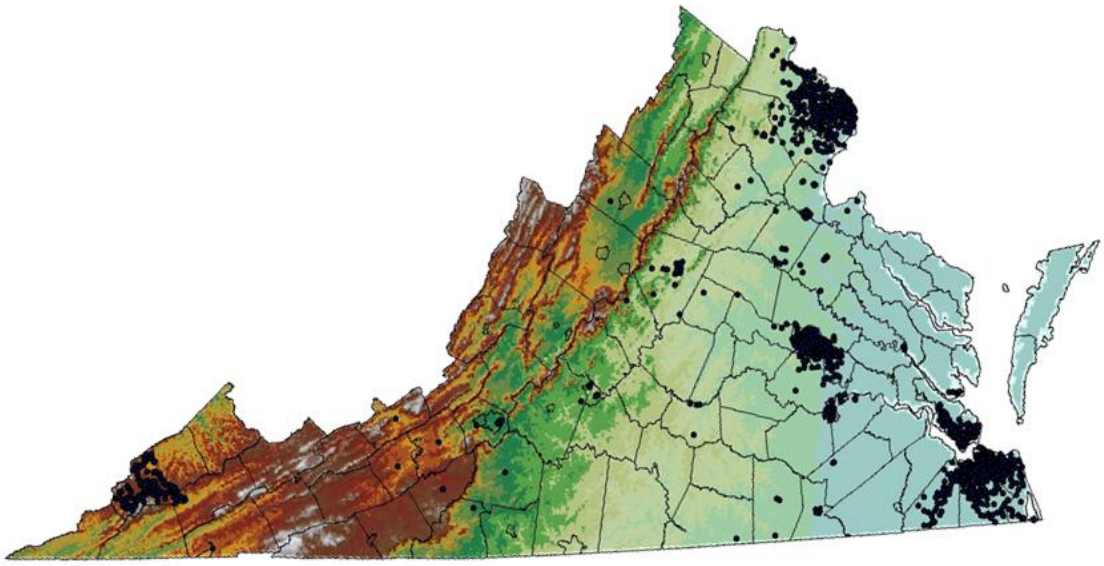

Figure 4.1. Location of the mosquito surveillance traps sites in Virginia from which the mosquito population data used in this study was collected. Trap sites are shown on an elevation map.

[2] Including all mosquito surveillance data gathered by mosquito control districts, from 2000-2006 a total of 2,788,036 mosquitoes were trapped over 239,252 trap nights. The mean number of female mosquitoes trapped per site per month was 15.32.

[3]The monthly mean mosquito index was calculated as the natural logarithm of the mean number of female mosquitoes trapped per site per month, multiplied by 10.

[4] A record is the monthly mean number of mosquitoes of a competent vector species trapped at a given site.

The majority of mosquitoes were collected in 2005 and 2006 (Table 4.3): 29.9% of the mosquitoes were collected in 2006 and 20.5% were collected in 2005. However, 2003 had the highest abundance index[3]; more mosquitoes were collected in 2003 (than other years) relative to the number of trap nights. Approximately 99% of mosquito collection occurred from April through October (Table 4.4), using a variety of traps (e.g., CDC light traps and gravid traps). The months with the highest percentage of mosquitoes collected were June (22.7%) and July (22.5%) followed by August (16.7%). April and May had the highest abundance indices of 1.77 and 1.21, respectively.

Table 4.3 Virginia competent vector mosquito surveillance statistics by year for 2000-2006.

Year	*Records*[b]	*# Mosquitoes*[a]	*# Trap Sites*	*# Trap nights*	*# Mosquitoes per Trap Night*	*Mean # Mosquitoes*	*Abundance Index*[c]
2000	848	90467	160	4094	22.10	27.15	2.10
2001	713	19212	82	4773	4.03	10.56	0.38
2002	1584	52094	379	3372	15.45	15.99	1.47
2003	2756	195631	334	7636	25.62	18.76	2.43
2004	5841	239563	527	22708	10.55	15.00	1.00
2005	8051	221544	701	29294	7.56	14.59	0.72
2006	9973	349620	800	38895	8.99	15.29	0.85

[a]The number of mosquitoes is the number of females trapped.
[b]A record is the monthly mean number of mosquitoes of a competent vector species trapped at a given site.
[c]The abundance indices were calculated using the following equation: [(number of female mosquitoes collected in the year) / (total number of female mosquitoes collected from 2000-2006] / [(number of trap nights in the year) / (total number of trap nights from 2000-2006)].

[3]The abundance index is a of the measure number of mosquitoes collected relative to the number of trap nights.

Table 4.4. Virginia competent vector mosquito surveillance statistics by month for 2000-2006.

Month	*Records*[b]	*# Mosquitoes*[a]	*# Trap Sites*	*# Trap Nights*	*# Mosquitoes per Trap Night*	*Mean # Mosquitoes*	*Abundance Index*[c]
January	10	140	7	10	14.00	20.60	1.33
March	4	10	4	4	2.50	11.50	0.24
April	699	28236	219	1517	18.61	16.10	1.77
May	3215	191518	573	12298	15.57	15.34	1.48
June	5225	264894	856	20790	12.74	16.52	1.21
July	5454	263157	975	21068	12.49	16.74	1.18
August	5900	195629	1168	23052	8.49	15.79	0.80
September	5170	155406	965	20025	7.76	14.68	0.74
October	3392	56548	688	10693	5.29	13.67	0.50
November	697	12593	125	1315	9.58	16.41	0.91

[a]The number of mosquitoes is the number of females trapped.
[b]A record is the monthly mean number of mosquitoes of a competent vector species trapped at a given site.
[c]The abundance indices were calculated using the following equation: [(number of female mosquitoes collected in the month) / (total number of female mosquitoes collected] / [(number of trap nights in the month) / (total number of trap nights)].

Table 4.5 and Figure 4.2 show the number of each competent vector species collected in Virginia. The greatest number of mosquitoes collected was *Cx. restuans* (16.96%), *Cx. salinarius* (15.57%), and *Cx. pipiens* (15.44%) followed by *Ae. canadensis* (15.08%), *Ae. vexans* (9.53%), *Ae. albopictus* (8.80%), *Ae. triseriatus* (6.44%), *Ae. taeniorhynchus* (2.29%), and *Ae. sollicitans* (0.58%). *Ae. canadensis* had the highest abundance index (2.47), followed by *Cx. pipiens* (1.20) and *Ae. taeniorhynchus* (1.20). Spatial maps showing trap site locations and mosquito density at the trap sites for each of the 9 mosquito species considered to be potential competent RVF vectors are displayed in Appendix A.

Table 4.5. Virginia competent vector mosquito surveillance statistics by mosquito species collected for 2000-2006.

Species	***Records*** [b]	***# Mosquitoes*** [a]	***# Trap Sites***	***# Trap Nights***	***# Mosquitoes per Trap Night***	***Mean # Mosquitoes***	***Abundance Index*** [c]
Ae. albopictus	5638	102797	1704	19601	5.24	13.77	0.49
Ae. canadensis	1655	176114	367	6772	26.01	15.50	2.47
Ae. sollicitans	248	6772	83	1366	4.96	8.91	0.47
Ae. taeniorhynchus	443	26695	156	2110	12.65	9.82	1.20
Ae. triseriatus	2797	75220	893	10998	6.84	10.15	0.65
Ae. vexans	5062	111321	1081	19976	5.57	12.67	0.53
Cx.. pipiens	4074	180325	1344	14286	12.62	20.28	1.20
Cx. pipiens/restuans [d]	1603	108893	533	4108	26.51	27.12	2.51
Cx. restuans	4465	198140	1068	16558	11.97	17.28	1.13
Cx. salinarius	3781	181854	810	14997	12.13	15.78	1.15

[a]The number of mosquitoes is number of females trapped.
[b]A record is the monthly mean number of mosquitoes of a competent vector species trapped at a given site.
[c]The abundance indices were calculated using the following equation: [(number of female mosquitoes of a given species collected) / (total number of female mosquitoes collected] / [(number of trap nights the species was collected) / (total number of trap nights)].
[d]For a significant number of trap records, the species was recorded as *Cx. pipiens/restuans*; this designation was used for *Cx. pipiens* and *Cx. restuans* collections which could not be morphologically distinguished. These records were included in competent vector group analyses because both species are considered potential competent vectors of RVF virus for this study.

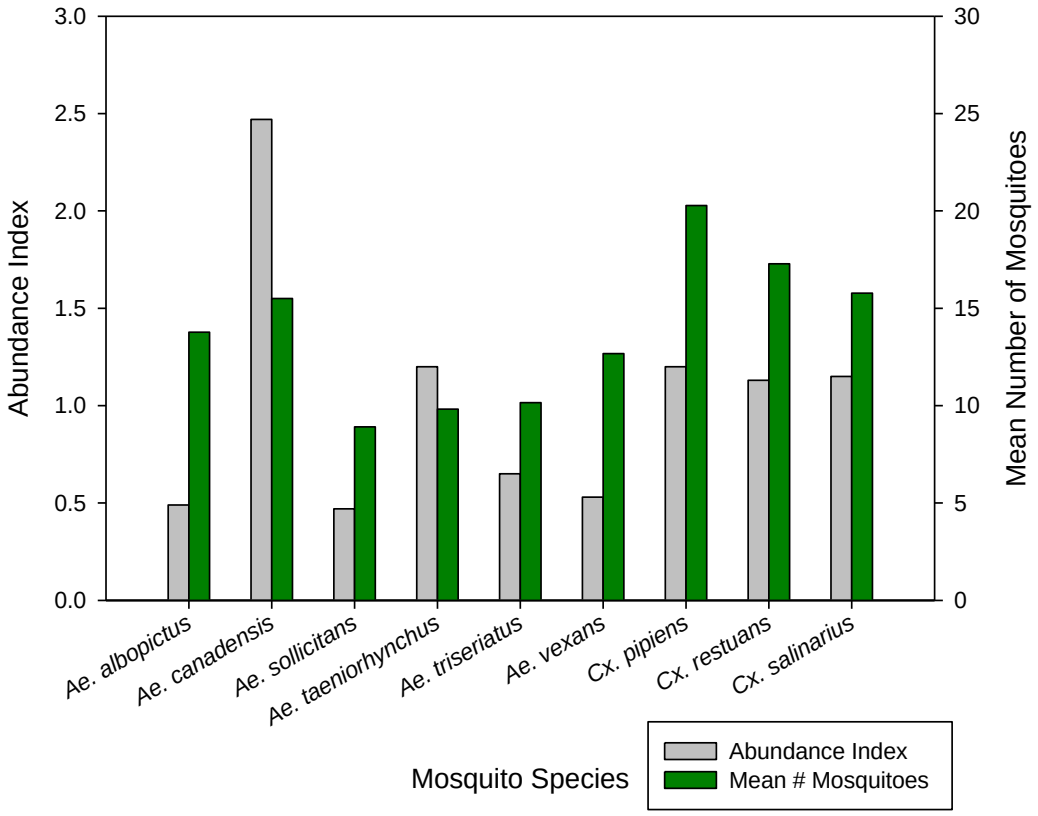

Figure 4.2. Abundance indices and mean number of mosquitoes of potential RVF competent vector mosquitoes. The abundance indices were calculated using the following equation: [(number of female mosquitoes of a given species collected) / (total number of female mosquitoes collected] / [(number of trap nights the species was collected) / (total number of trap nights)].

Ninety percent of the mosquito surveillance data for Virginia were collected in the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions of the state (Table 4.6). The greatest number of mosquitoes were collected in Hampton Roads (49%) followed by Northern Virginia (30%) and Richmond-Petersburg (11%). The Richmond-Petersburg region had the highest abundance index (1.46).

Table 4.6. Virginia competent vector mosquito surveillance statistics by region for 2000-2006.

Region	*Records*[b]	*# Mosquitoes*[a]	*# Trap Sites*	*# Trap Nights*	*# Mosquitoes per Trap Night*	*Mean # Mosquitoes*	*Abundance Index*[c]
Northern Virginia	8835	352791	447	38473	9.17	15.74	0.87
Richmond-Petersburg	4268	128966	483	8376	15.40	19.75	1.46
Hampton Roads	14951	573866	771	61156	9.38	14.04	0.89

[a]The number of mosquitoes is the number of females trapped.
[b]A record is the monthly mean number of mosquitoes of a competent vector species trapped at a given site
[c]The abundance indices were calculated using the following equation: [(total number of female mosquitoes collected in the region) / (total number of female mosquitoes collected in Virginia] / [(number of trap nights in the region) / (total number of trap nights in Virginia)].

In the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions, the majority of the mosquitoes collected were of the genus *Culex* (55.7%) followed by *Aedes* (44.3%). The greatest number of competent vectors collected was the species *Cx. pipiens* (20.6%), followed by *Ae. canadensis* (19.8%), *Cx. restuans* (18.2%, *Cx. salinarius* (17.0%), *Ae. vexans* (9.7%), *Ae. albopictus* (9.6%), *Ae. taeniorhynchus* (3.7%), and *Ae. triseriatus* (0.9%), and *Ae. sollicitans* (0.6%).

In Northern Virginia the largest percentage of competent vector mosquitoes collected was the genus *Culex* (83.6%), followed by *Aedes* (16.3%). Analyzed by species, the largest percentage of mosquito species collected was *Cx. restuans* (49.3%), followed by *Cx. pipiens* (32.6%), *Ae. vexans* (8.5%), *Ae. albopictus* (6.6%), *Ae. triseriatus* (1.3%), *Cx. salinarius* (1.1%), *Ae. canadensis* (0.5%), *Ae. sollicitans* (0.0%), and *Ae. taeniorhynchus* (0.0%).

As in Northern Virginia, the largest percentage of competent vectors collected in Richmond-Petersburg was of the genus *Culex* (71.6%), followed by *Aedes* (28.4%). The

majority of the mosquito species collected were *Cx. pipiens* (36.8%) and *Ae. albopictus* (31.4%), followed by *Ae. vexans* (13.0%), *Cx. restuans* (11.5%), *Cx. salinarius* (3.1%), *Ae. canadensis* (2.1%), *Ae. triseriatus* (2.0%), *Ae. taeniorhynchus* (0.1%), and *Ae. sollicitans* (0.0%).

In the Hampton Roads region, the largest percentage of competent vectors collected was of the genus *Aedes* (51.1%), followed by *Culex* (48.9%). The majority of the mosquito species collected was *Ae. canadensis* (32%), followed by *Cx. salinarius* (26.9%), *Cx. pipiens* (11.9%), *Ae. vexans* (9.5%), *Ae. albopictus* (6.2%), *Cx. restuans* (6.1%), *Ae. taeniorhynchus* (6.1%), *Ae. sollicitans* (0.9%), and *Ae. triseriatus* (0.5%).

Twenty-two percent of all mosquitoes collected in Virginia were collected in Chesapeake City. Fairfax County (18.23%) and Suffolk City (8.56%) collected the next highest number of potential RVF competent vectors. Wise county had the highest abundance index (12.10) followed by Prince Edward County (10.23) and Roanoke City (7.89).

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Table 4.7. Virginia competent vector mosquito surveillance statistics by county for 2000-2006

County	*Records*[b]	*# Mosquitoes*	*# Trap Sites*	*# Trap Nights*	*# Mosquitoes per Trap Night*	*Mean # Mosquitoes*	*Abundance Index*[c]
Albemarle County	50	457	18	50	9.14	18.80	0.87
Alexandria City	580	28603	34	3535	8.09	14.16	0.77
Arlington County	1204	48528	70	4275	11.35	16.01	1.08
Bedford County	3	20	1	3	6.67	17.67	0.63
Bristol City	2	15	1	2	7.50	20.50	0.71
Brunswick County	6	12	2	6	2.00	9.67	0.19
Caroline County	91	10929	12	187	58.44	25.93	5.54
Charlottesville City	182	4246	24	304	13.97	19.72	1.32
Chesapeake City	4290	266225	111	14883	17.89	15.11	1.70
Chesterfield County	142	1519	25	179	8.49	17.27	0.80
Colonial Heights City	9	99	4	9	11.00	17.33	1.04
Culpeper County	7	34	2	7	4.86	11.86	0.46
Emporia City	43	949	8	95	9.99	16.09	0.95
Fairfax City	298	18168	9	1378	13.18	14.27	1.25
Fairfax County	5932	212984	220	26193	8.13	14.05	0.77
Falls Church City	3	28	1	12	2.33	9.00	0.22
Fauquier County	42	673	11	60	11.22	17.88	1.06
Floyd County	17	185	2	32	5.78	15.94	0.55
Fluvanna County	7	31	1	7	4.43	14.57	0.42
Franklin County	7	22	3	7	3.14	10.57	0.30
Fredericksburg City	105	1865	29	110	16.95	22.57	1.61
Giles County	18	164	1	38	4.32	13.67	0.41
Gloucester County	27	1236	6	48	25.75	21.44	2.44
Goochland County	14	862	3	14	61.57	23.93	5.84
Hampton City	517	14340	27	1699	8.44	12.81	0.80
Hanover County	42	494	11	51	9.69	17.79	0.92
Henrico County	2819	63986	327	4972	12.87	19.55	1.22
Hopewell City	37	450	12	37	12.16	20.57	1.15
King George County	4	16	2	5	3.20	13.25	0.30
King William County	15	141	4	15	9.40	18.73	0.89
Loudoun County	328	6221	56	912	6.82	14.58	0.65
Lynchburg City	17	145	7	17	8.53	17.18	0.81
Mecklenburg County	2	26	1	2	13.00	21.50	1.23
Montgomery County	68	1280	7	194	6.60	16.60	0.63
Nelson County	5	18	1	5	3.60	14.80	0.34
Newport News City	257	3474	101	319	10.89	18.25	1.03
Norfolk City	4303	98042	340	21617	4.54	13.51	0.43
Patrick County	2	10	1	2	5.00	16.50	0.47

Petersburg City	296	11600	27	461	25.16	21.35	2.39
County	***Records***[b]	***# Mosquitoes***	***# Trap Sites***	***# Trap Nights***	***# Mosquitoes per Trap Night***	***Mean # Mosquitoes***	***Abundance Index***[c]
Portsmouth City	707	39820	29	2405	16.56	14.76	1.57
Prince Edward County	52	6259	5	58	107.91	25.50	10.23
Prince George County	1	1	1	1	1.00	7.00	0.09
Prince William County	490	38259	57	2168	17.65	17.39	1.67
Pulaski County	86	1510	5	187	8.07	15.35	0.77
Richmond City	997	50999	101	2750	18.55	20.13	1.76
Roanoke City	21	1748	8	21	83.24	28.48	7.89
Roanoke County	180	3417	39	268	12.75	19.02	1.21
Rockingham County	7	168	2	7	24.00	22.29	2.28
Spotsylvania County	34	448	8	34	13.18	18.12	1.25
Stafford County	25	109	9	25	4.36	14.20	0.41
Suffolk City	2517	100031	56	13050	7.67	12.41	0.73
Sussex County	8	57	2	8	7.13	18.625	0.68
Virginia Beach City	2360	51934	107	7183	7.23	14.43	0.69
Wise County	441	73631	143	577	127.61	41.27	12.10
York County	49	1643	7	288	5.70	12.92	0.54

[a]The number of mosquitoes is the number of females trapped.
[b]A record is the monthly mean number of mosquitoes of a competent vector species trapped at a given site.
[c]The abundance indices were calculated using the following equation: [(total number of female mosquitoes collected in the county) / (total number of female mosquitoes collected in Virginia] / [(number of trap nights in the county) / (total number of trap nights in Virginia)].

4.3 Mosquito Density Correlations with Environmental-Climatic Variables

The results presented here were obtained from analyses of mosquito surveillance data collected between April and October for 2005-2006 at the Northern Virginia, Richmond-Petersburg, and Hampton Roads trap sites shown in Figure 4.3. Mosquito surveillance data from these traps were collapsed to create records representing the monthly average number of competent vectors collected per trap site. A total of 1,443 records were included in Northern Virginia analyses; 2,525 and 773 records were included in analyses of the Hampton Roads and Richmond-Petersburg regions, respectively. The 2005-2006 mean and range recorded for each environmental-climatic predictor variable used in regional correlation analyses are summarized in Table 4.8.

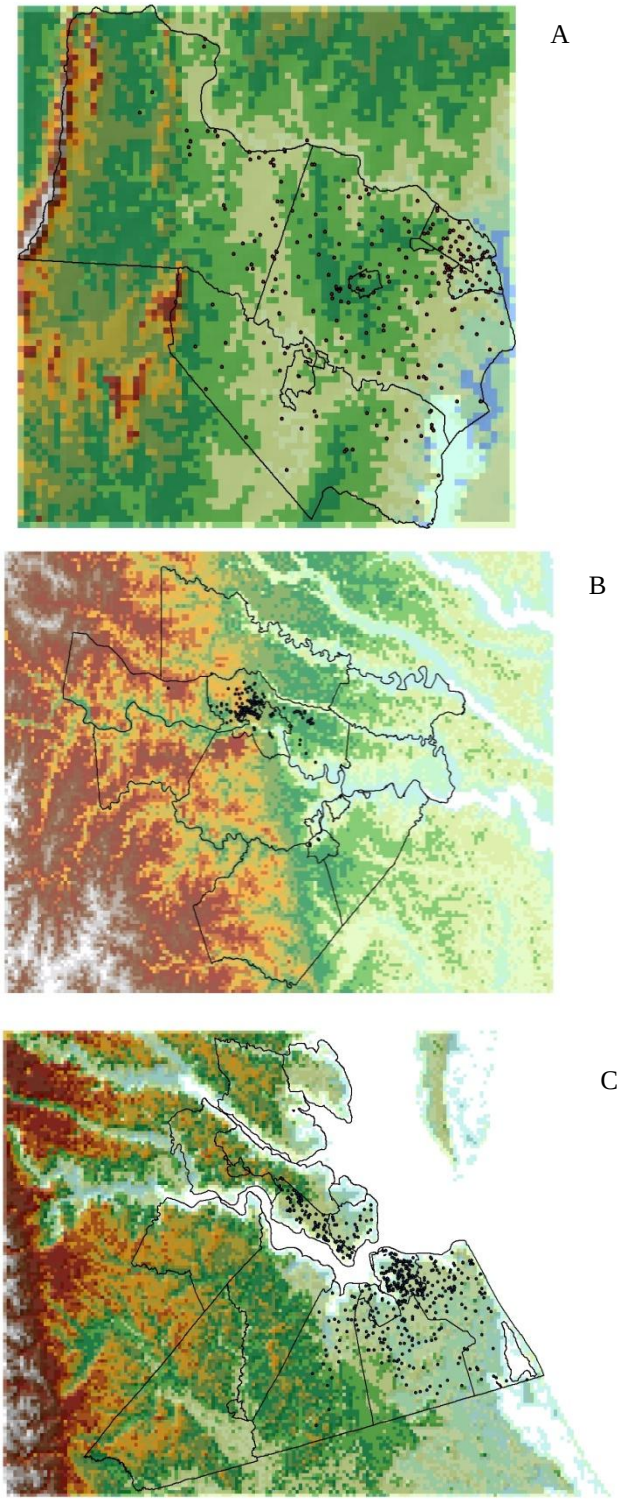


Figure 4.3. Mosquito surveillance trap locations in 2005-2006 in the Northern Virginia (A), Richmond-Petersburg (B), and Hampton Roads (C) regions of Virginia.

Table 4.8. Mean and range of each variable for the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions. The first set of values is associated with the pixels (1 km x 1km) within the region for the 2005-2006 time period. The second set of values (in bold text) is associated with the regional April-October trap records for the 2005-2006 time period.

Variable	***Description***	***Northern Virginia Mean (Range)***	***Richmond-Petersburg Mean (Range)***	***Hampton Roads Mean (Range)***
Sample size	n = number of pixels	6973	21824	17576
	n = number of records	**1443**	**773**	**2525**
avgfem	Average monthly number of females per trap (Apr-Oct)	**15.37 (1.00-73.00)**	**20.55 (7.00-49.00)**	**14.36 (1.00-47.00)**
NDVI_m	Average monthly NDVI	0.677 (0.278-0.837)	0.695 (0.099-0.832)	0.642 (-1.000-0.922)
		0.630 (0.418-0.808)	**0.607 (0.438-0.738)**	**0.513 (0.590-0.838)**
NDVI_lt[a]	Long-term average monthly NDVI	0.696 (0.443-0.800)	0.718 (0.120-0.827)	0.667 (-1-0.852)
		0.663 (0.504-0.831)	**0.653 (0.480-0.776)**	**0.537 (0.0807-0.852)**
tmin_m	Average monthly minimum temperature, °C	13.22 (4.06-21.57)	13.99 (4.01-22.52)	15.30 (5.42-23.13)
		15.44 (5.25-21.57)	**16.36 (5.71-22.44)**	**15.83 (-1.43-23.13)**
tmin_lt[b]	Long-term average monthly minimum temperature, °C	12.73 (0.00-13.90)	13.34 (0.00-20.80)	14.37 (0.00-21.70)
		14.48 (5.20-19.70)	**15.39 (6.20-19.30)**	**16.89 (0.00-21.70)**
tmax_m	Average monthly maximum temperature, °C	25.18 (16.43-31.93)	26.85 (18.45-33.11)	26.89 (18.96-33.09)
		26.73 (17.81-31.84)	**28.58 (19.73-32.81)**	**26.95 (19.17-32.37)**
tmax_lt[b]	Long-term average monthly maximum temperature, °C	25.17 (0.00-26.14)	26.28 (0.00-32.00)	26.18 (0.00-32.00)
		26.75 (18.50-31.2)	**28.06 (20.60-31.80)**	**26.51 (0.00-30.80)**
ppt_m	Average monthly precipitation, mm	100.75 (4.04-269.03)	104.42 (1.82-274.99)	112.16 (-327.60-324.17)
		105.56 (7.20-10.50)	**103.93 (5.58-219.26)**	**120.96 (34.14-290.51)**
ppt_lt[b]	Long-term average monthly precipitation, mm	90.49 (0.00-101.00)	96.06 (0.00-132.00)	99.34 (0.00-149.00)
		91.30 (72.00-105.00)	**99.24 (77.00-117.00)**	**10.45 (0.00-14.20)**
altitude	Elevation above sea level, m	119.14 (-11.00-533.00)	68.17 (-3.00-212.00)	24.93 (-10.00-103.00)
		67.47 (0.00-181.00)	**68.21 (22.00-105.00)**	**7.76 (-1.00-34.00)**
bio 01[b]	Annual mean temperature, °C	124.14 (0.00-135.00)	138.49 (0.00-149.00)	145.00 (0.00-158.00)
		127.85 (112-134.00)	**138.07 (134.00-143.00)**	**152.09 (0.00-155.00)**
bio 02[b]	Mean diurnal range (Mean of monthly (max temp - min temp)), °C	121.48 (104.00-134.00)	126.92 (103.00-136.00)	117.81 (94.00-134.00)
		118.50 (114.00-126.00)	**127.46 (126.00-230.00)**	**99.04 (94.00-117.00)**
bio 03[b]	Isothermality ((bio2 / bio7)*100), °C	33.52 (30.00-36.00)	36.17 (31.00-38.00)	35.07 (30.00-38.00)
		33.04 (32.00-35.00)	**36.04 (36.00-37.00)**	**31.71 (30.00-36.00)**
bio 04[b]	Temperature seasonality (standard deviation *100), °C	8487.39 (8255.00-8633.00)	7951.30 (7598.00-8151.00)	7746.75 (7205.00-8079.00)
		8486.39 (8367.00-8585.00)	**8021.36 (7922.00-8062.00)**	**7581.74 (7308.00-7841.00)**

Variable	*Description*	*Northern Virginia Mean (Range)*	*Richmond-Petersburg Mean (Range)*	*Hampton Roads Mean (Range)*
bio 05[b]	Max temperature of warmest month, °C	303.84 (272.00-313.00) **305.23 (301-312)**	310.46 (300.00-320.00) **311.27 (308.00-318.00)**	310.20 (298.00-320.00) **305.09 (300.00-308.00)**
bio 06[b]	Min temperature of coldest month, °C	-53.20 (-74.00- -36.00) **-47.58 (-58.00- -38.00)**	-35.46 (-47.00- -14.00) **-37.74 (-44.00- -31.00)**	-20.53 (-39.00- 5.00) **-2.96 (-17.00- 2.00)**
bio 07[b]	Temperature annual range (bio5 – bio6), °C	357.05 (341.00-367.00) **352.81 (345.00-363.00)**	345.92 (315.00-356.00) **349.01 (345.00-352.00)**	330.74 (297.00-352.00) **308.043 (299.00-324.00)**
bio 08[b]	Mean temperature of wettest quarter , °C	217.90 (189.00-243.00) **229.07 (207.00-243.00)**	236.20 (223.00-246.00) **234.81 (230.00-340.00)**	240.57 (231.00-247.00) **243.56 (239.00-246.00)**
bio 09[b]	Mean temperature of driest quarter, °C	11.53 (-13.00-25.00) **15.44 (6.00-24.00)**	62.98 (22.00-106.00) **34.30 (27.00-96.00)**	100.86 (31.00-162.00) **112.05 (103.00-118.00)**
bio 10[b]	Mean temperature of warmest quarter, °C	231.09 (0.00-243.00) **235.07 (218.00-242.00)**	238.60 (0.00-246.00) **239.38 (235.00-244.00)**	241.82 (0.00-251.00) **247.68 (0.00-251.00)**
bio 11[b]	Mean temperature of coldest quarter, °C	11.51 (-14.00-26.00) **15.43 (-1.00-24.00)**	33.16 (0.00-47.00) **31.64 (27.00-38.00)**	43.54 (0.00-64.00) **53.06 (0.00-59.00)**
bio 12[b]	Annual precipitation, mm	1018.00 (0.00-1102.00) **1013.09 (984.00-1079.00)**	1102.39 (0.00-1198.00) **1095.18 (1081.00-1113.00)**	1133.16 (0.00-1227.00) **1144.17 (0.00-59.00)**
bio 13[b]	Precipitation of wettest month, mm	101.73 (95.00-111.00) **101.88 (97.00-105.00)**	113.09 (104.00-132.00) **110.24 (106.00-118.00)**	125.15 (104.00-150.00) **130.36 (120.00-142.00)**
bio 14[b]	Precipitation of driest month, mm	68.92 (63.00-75.00) **69.91 (66.00-72.00)**	77.39 (70.00-83.00) **77.17 (76.00-80.00)**	75.81 (70-81) **73.12 (71-75)**
bio 15[b]	Precipitation seasonality (coefficient of variation), mm	11.67 (9.00-15.00) **11.11 (10.00-12.00)**	11.76 (8.00-18.00) **11.21 (10.00-13.00)**	15.38 (11.00-25.00) **18.11 (71.00-75.00)**
bio 16[b]	Precipitation of wettest quarter, mm	287.38 (272.00-314.00) **285.41 (272.00-295.00)**	316.00 (295.00-368.00) **311.03 (299.00-326.00)**	345.72 (293.00-409.00) **362.19 (336.00-386.00)**
bio 17[b]	Precipitation of driest quarter, mm	216.04 (195.00-240.00) **220.60 (208.00-226.00)**	246.05 (225.00-261.00) **249.21 (244.00-252.00)**	243.72 (225.00-257.00) **238.08 (227.00-248.00)**
bio 18[b]	Precipitation of warmest quarter, mm	285.15 (271.00-302.00) **284.41 (271.00-295.00)**	314.77 (293.00-368.00) **309.92 (299.00-321.00)**	342.36 (290.00-400.00) **352.78 (331.00-376.00)**
bio 19[b]	Precipitation of coldest quarter, mm	216.04 (195.00-240.00) **220.60 (208.00-226.00)**	252.48 (225.00-280.00) **249.49 (244.00-257.00)**	265.76 (246.00-286.00) **266.38 (256.00-282.00)**

[a] NOAA AVHRR 1981-2005 NDVI.
[b] Worldclim 1950-2000 climate data.

Regional graphical analyses

In Figures 4.4-4.6, mosquito populations are plotted with the Normalized Difference Vegetation Index (NDVI), temperature, and precipitation for the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions. Figure 4.7 shows the change in mosquito populations between 2005 and 2006 relative to change in NDVI, temperature, and precipitation between 2005 and 2006

It is evident in Figure 4.4 that the monthly mean number of mosquitoes collected at Northern Virginia trap sites was associated with NDVI and maximum and minimum temperature. Monthly mean mosquito counts increased with increasing long-term and monthly mean NDVI values and increasing monthly mean maximum and minimum temperatures. Anomaly NDVI values provide a measure of positive or negative deviation from the mean NDVI value for each month. Negative values relative to the anomaly NDVI zero line indicate lower-than-average greenness (dry periods) from April through October in both 2005 and 2006. The affect of precipitation was not as clear, however, monthly mosquito counts tended to increase following months with greater mean precipitation.

For the Richmond-Petersburg and Hampton Roads regions, an association between mosquito density and NDVI and temperature (Figures 4.5 and 4.6) was not as apparent as in the Northern Virginia region. Similar to Northern Virginia analyses, negative anomaly NDVI values indicate that both the Richmond-Petersburg and Hampton Roads regions experienced lower-than-average greenness (dry periods) from April through October in both 2005 and 2006. In addition, months with greater precipitation tended to precede increased monthly mosquito counts.

In Northern Virginia, mosquito populations, NDVI, and temperature were higher in 2006 than in 2005 for all months except September (Figure 4.7). In the Richmond-Petersburg region, mosquito populations were lower in 2006 than in 2005 for all months except April and May. In the Hampton Roads region, mosquito populations were higher in 2006 than in 2005 for July, August, September, and October. In all three regions winter temperatures and NDVI values in 2006 were generally greater than in 2005 while winter precipitation in 2006 was lower than in 2005. During the spring and summer months when mosquito populations are typically highest, the conditions were reversed.

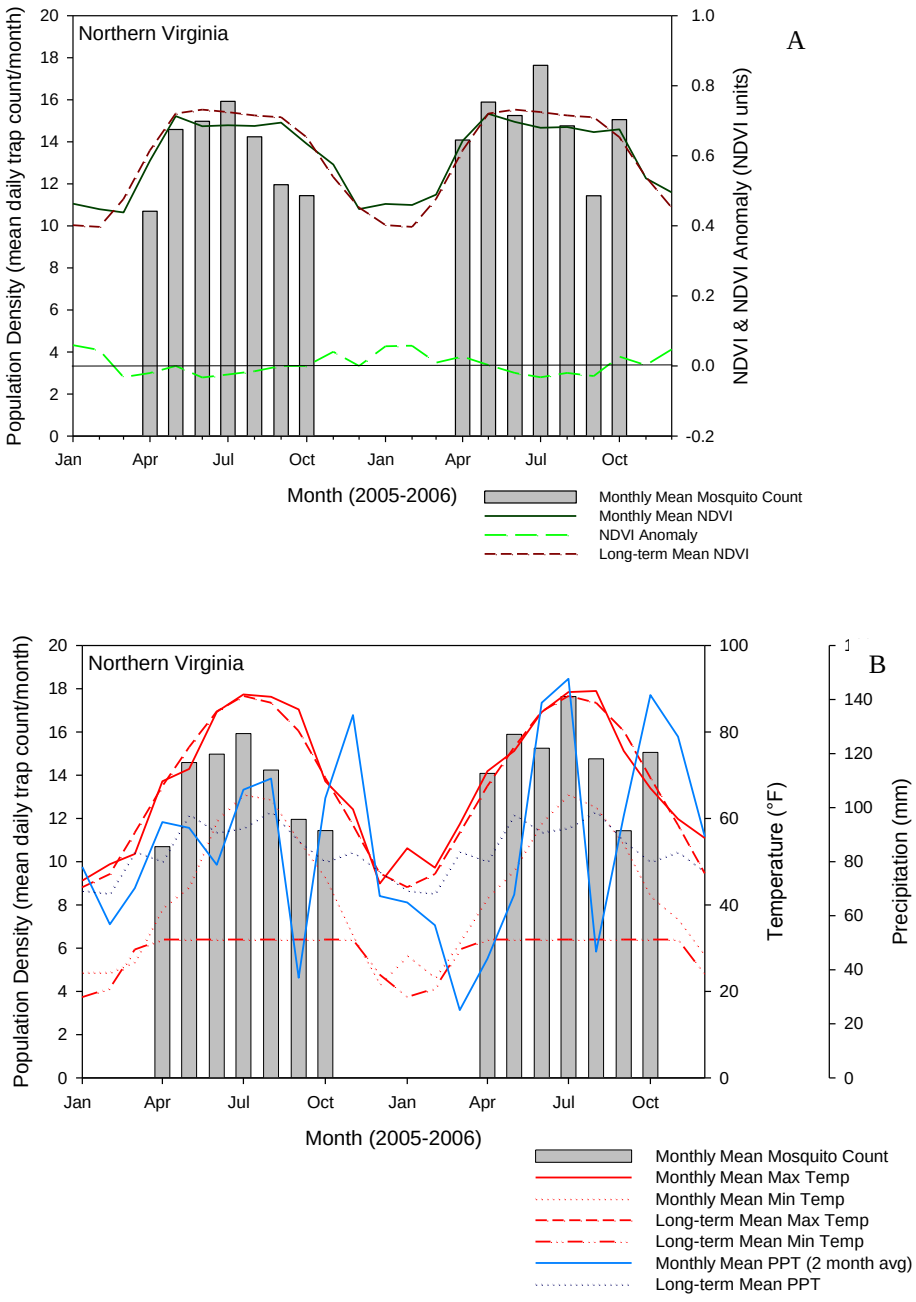


Figure 4.4. Monthly mean number of RVF competent vectors collected per month for 2005-2006 in the Northern Virginia region. In Figure A the mosquito counts are plotted with monthly and long-term mean NDVI and NDVI anomaly. In Figure B, the mosquito counts are plotted with monthly mean and long-term mean minimum and maximum temperature and monthly and long-term mean precipitation.

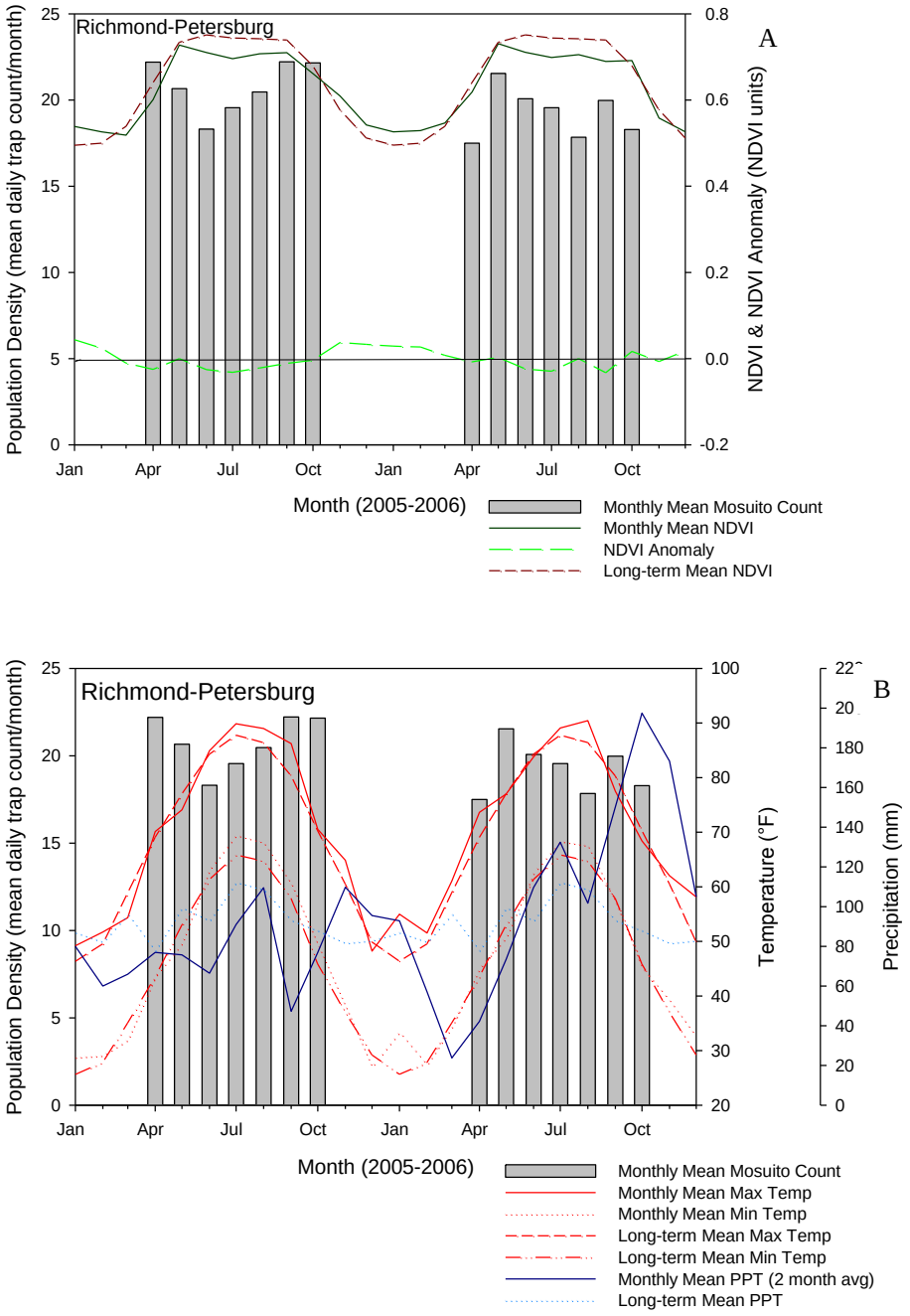


Figure 4.5. Monthly mean number of RVF competent vectors collected per month for 2005-2006 in the Hampton Roads region. In Figure A the mosquito counts are plotted with monthly and long-term mean NDVI and NDVI anomaly. In Figure B, the mosquito counts are plotted with monthly mean and long-term mean minimum and maximum temperature and monthly and long-term mean precipitation.

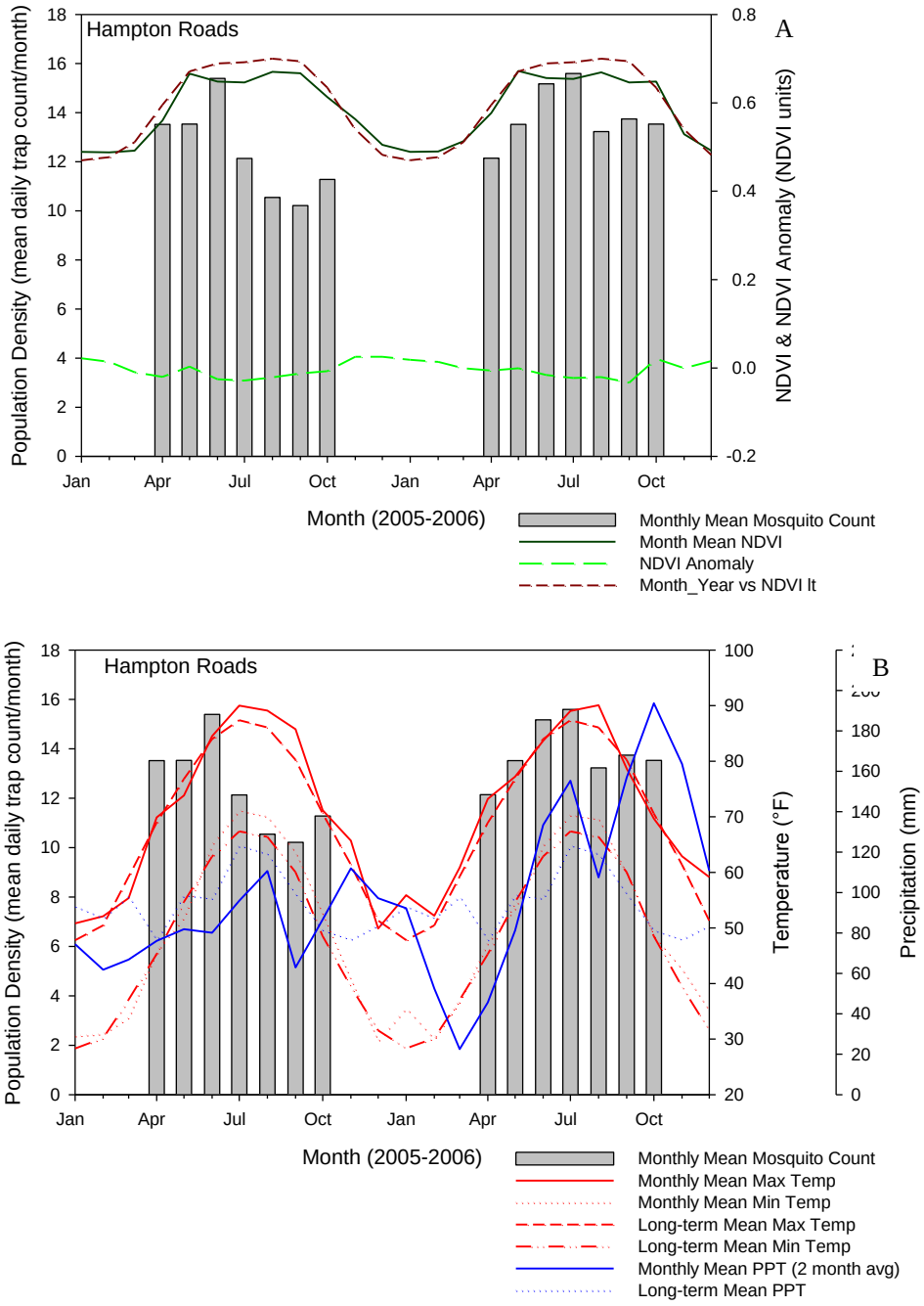


Figure 4.6. Monthly mean number of RVF competent vectors collected per month for 2005-2006 in the Hampton Roads region. In Figure A the mosquito counts are plotted with monthly and long-term mean NDVI and NDVI anomaly. In Figure B, the mosquito counts are plotted with monthly mean and long-term mean minimum and maximum temperature and monthly and long-term mean precipitation.

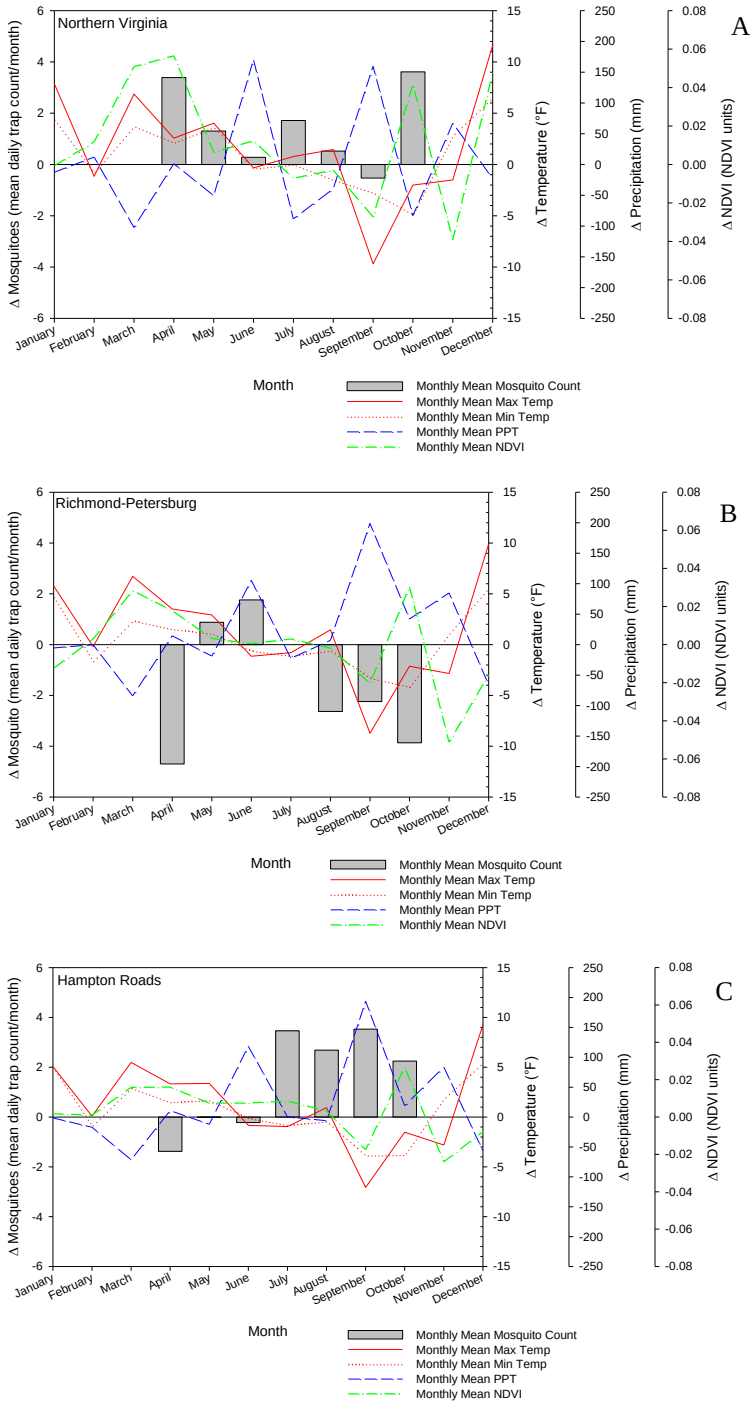


Figure 4.7. Difference in 2005 and 2006 monthly mean mosquito counts in the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions. The difference in 2005 and 2006 monthly mean mosquito counts is represented by the vertical bars. The difference in 2005 and 2006 mean maximum and minimum temperature (red), monthly mean precipitation (blue), and monthly mean NDVI (green) are also shown.

Competent vector species were plotted with precipitation (Figure 4.8-Figure 4.10) to visualize seasonal temporal trends of each species and to evaluate whether precipitation in each region affects competent vector species populations differently. In 2006, *Cx. restuans* populations were greatest in early-mid spring in all three regions, while *Cx. pipiens* populations generally peaked in mid-summer. *Ae. vexans* and *Ae. albopictus* populations peaked late in the summer. In the Richmond-Petersburg and Hampton Roads regions, precipitation in 2006 was greatest in August through October. During these months, *Cx. pipiens* populations were lower when precipitation was higher, while *Ae. vexans* and *Ae. canadensis* populations increased with increasing precipitation.

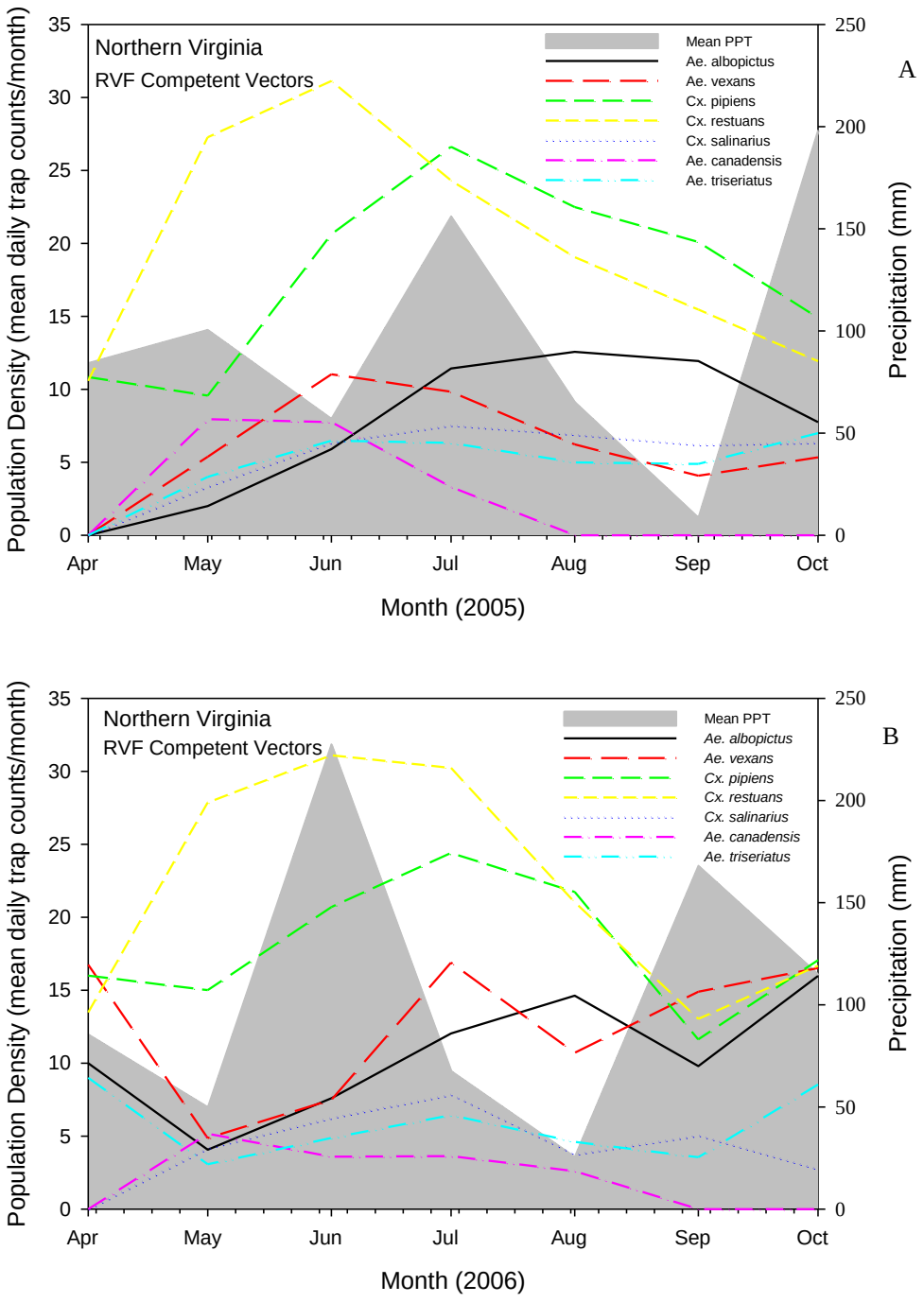


Figure 4.8. Monthly mean number of RVF competent vector species collected in 2005 (A) and 2006 (B) during mosquito surveillance months (April-October) in the Northern Virginia region. Monthly mean precipitation for the same time period is shown in gray.

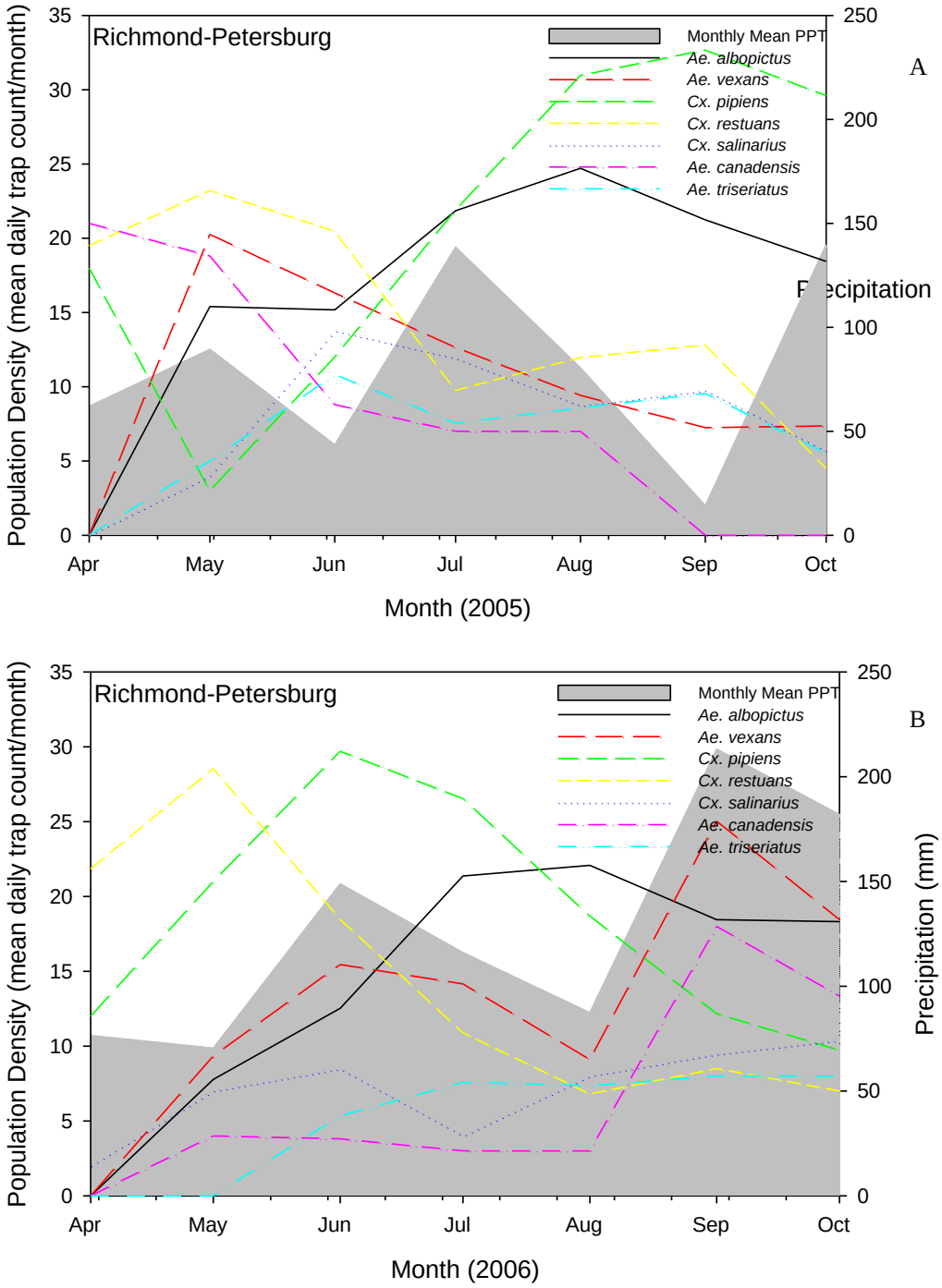
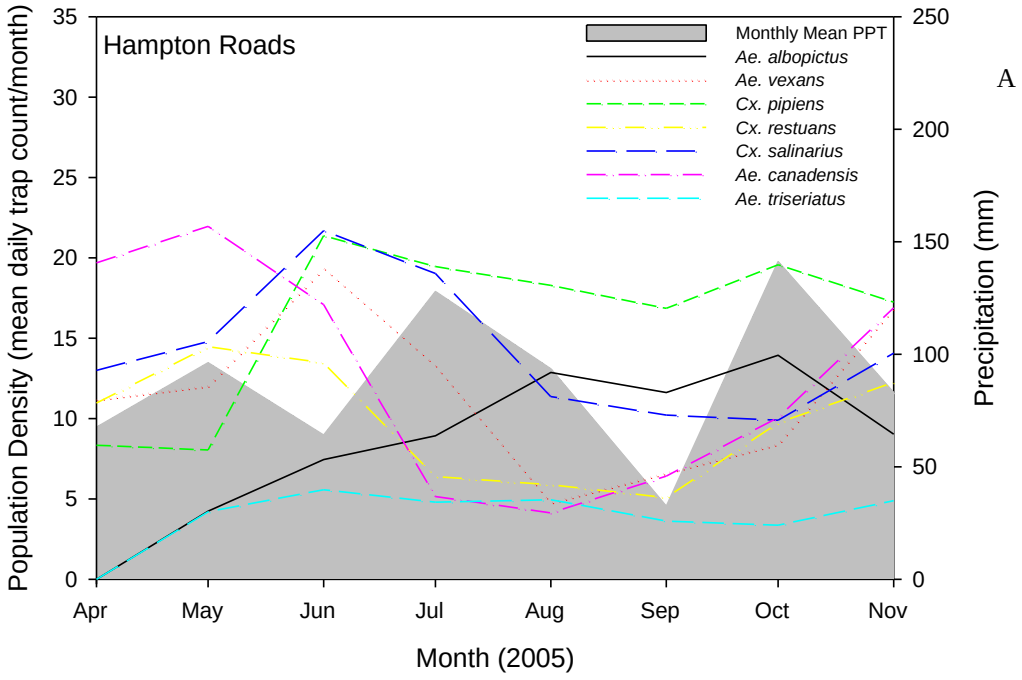


Figure 4.9. Monthly mean number of RVF competent vector species collected in 2005 (A) and 2006 (B) during mosquito surveillance months (April-October) in the Richmond-Petersburg region. Monthly mean precipitation for the same time period is shown in gray.



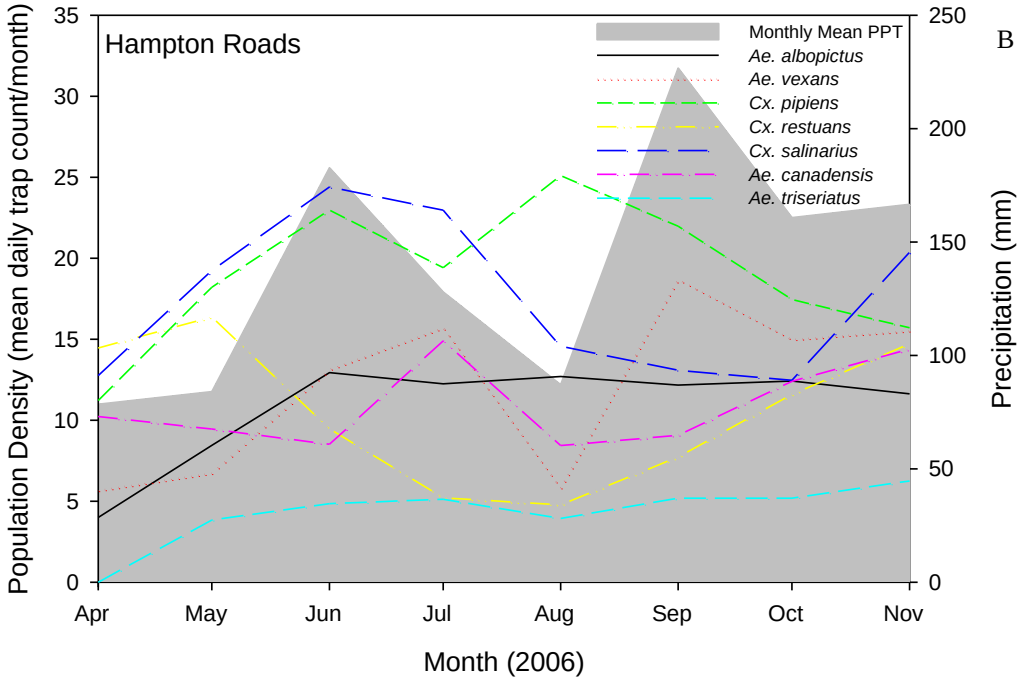


Figure 4.10. Monthly mean number of RVF competent vector species collected in 2005 (A) and 2006 (B) during mosquito surveillance months (April-October) in the Hampton Roads region. Monthly mean precipitation for the same time period is shown in gray

Predictor correlation analyses

While Figures 4.4-4.10 indicate there may be some relationship between mosquito abundance and distribution and the environmental-climatic variables included in these analyses, further analysis was required to better evaluate the nature of this relationship. The Pearson Product Moment Correlation was used to determine if there are any significant correlations between the mosquito collection data and environmental-climatic variables. The summary report of the Pearson Product Moment Correlation (Table 4.9) displays the correlation coefficient (r), the P-value for the correlation coefficient, and the number of data points used in the computation, for each pair of variables. The correlation coefficient quantifies the strength of the association between the variables and varies between -1 and +1. The closer a correlation coefficient is to +1 or -1, the higher the correlation between the two variables or the stronger the relationship. A correlation coefficient near +1 indicates there is a strong positive relationship between the two variables, with both always increasing together where as a correlation coefficient near -1 indicates there is a strong negative relationship between the two variables, with one always decreasing as the other increases. A correlation coefficient of 0 indicates no relationship between the two variables. The P-value is the probability of being wrong in concluding that there is a true association between the variables (i.e., the probability of falsely rejecting the null hypothesis). The smaller the P-value, the greater the probability that the variables are correlated. The results of these analyses indicated that some environmental-climatic (independent) variables can be used to predict the mosquito population (dependent) variable when $P < 0.05$.

For the ***Northern Virginia*** region, extracting values by trap for April-October 2005-2006 mosquito surveillance data, the number of data points for which associations were

measured was 1,443. Statistical analyses revealed significant positive correlations between the monthly mean competent vector population and monthly mean NDVI, long-term mean NDVI, minimum and maximum temperature, long-term mean minimum and maximum temperature (Table 4.9). There is also a positive correlation between the average monthly competent vector population and the following bioclimatic variables: annual mean temperature (bio 01), minimum temperature of the coldest month (bio 06), mean temperature of the driest quarter (bio 09), mean temperature of the coldest quarter (bio 11), and precipitation of the driest month (bio 14). Statistical analyses revealed a significant inverse correlation between the monthly mean competent vector population and the following bioclimatic variables: temperature seasonality (bio 04), temperature annual range (bio 07), annual precipitation (bio 12), and precipitation seasonality (bio 15). NDVI is correlated with elevation ($r = -0.0452$, $p < 0.05$) in the Northern Virginia region. Precipitation is inversely correlated with mean maximum ($r = -0.0313$, $p < 0.05$) and minimum temperature ($r = -0.171$, $p < 0.05$). For variable pairs with P values greater than 0.05 there is no significant relationship between the two variables.

In summary, in Northern Virginia there is a direct relationship between mosquito collection data and both NDVI and temperature data. Mosquito populations tend to increase with NDVI and temperature. NDVI is greater at lower elevations.

For the ***Richmond-Petersburg*** region, extracting values by trap for April-October 2005-2006 mosquito surveillance data, the number of data points for which associations were measured was 773. Statistical analyses revealed significant positive correlations between the monthly mean competent vector population and the following bioclimatic variables: annual mean temperature (bio 01), maximum temperature of the warmest month (bio 05), minimum

temperature of the coldest month (bio 06), mean temperature of the warmest quarter (bio 10), mean temperature of the coldest quarter (bio 11), precipitation of the wettest month (bio 13), precipitation seasonality (bio 15), precipitation of the wettest quarter (bio 16), precipitation of the warmest quarter (bio 18), and precipitation of the coldest quarter (bio 19). There is a negative correlation between the average monthly competent vector population and elevation, long-term mean monthly maximum and minimum temperature, long-term mean monthly maximum NDVI, and mean temperature of the wettest quarter (bio 08). For variable pairs with P values greater than 0.05 there is no significant relationship between the two variables.

Mean minimum temperatures in the Richmond-Petersburg region are greater at lower elevations ($r = -0.0903$, $p < 0.05$). Although precipitation is not correlated with elevation ($r = 0.0139$, $p = 0.700$), precipitation is correlated with both mean maximum temperature ($r = -0.382$, $p < 0.05$) and mean minimum temperature ($r = -0.222$, $p < 0.05$). NDVI and elevation are correlated ($r = -0.199$, $p < 0.05$); NDVI values are greater at lower elevations. There is no significant relationship between NDVI and temperature or NDVI and precipitation.

In summary, as in the Northern Virginia region, NDVI is greater at lower elevations in the Richmond-Petersburg region. There is no significant relationship between mosquito collection data and monthly NDVI data in this region, however there is a significant relationship between mosquito collection data and elevation. Mosquito populations tended to be greater at lower elevations (greater NDVI values). There is a direct positive relationship between mosquito collection data and precipitation data. Mosquito populations tend to increase with precipitation. There is a significant inverse correlation between mosquito collection data and temperature; mosquito populations tend to be greater when temperature is lower and precipitation is greater.

For the ***Hampton Roads*** region, extracting values by trap for April-October 2005-2006 mosquito surveillance data, the number of data points for which associations were measured was 2,525. Statistical analyses revealed significant positive correlations between the monthly mean competent vector population and monthly mean precipitation, long-term mean monthly minimum temperature, annual mean temperature (bio 01), temperature seasonality (bio 04), mean temperature of the wettest quarter (bio 08), mean temperature of the driest quarter (bio 09), mean temperature of the warmest quarter (bio 10), mean temperature of the coldest quarter (bio 11), and precipitation seasonality (bio 15). There is a negative correlation between the average monthly competent vector population and elevation, monthly mean maximum NDVI, long-term mean monthly maximum NDVI, mean diurnal range (bio 02), isothermality (bio 03), maximum temperature of the warmest month (bio 05), temperature annual range (bio 07), annual precipitation (bio 12), precipitation of the wettest month (bio 13), precipitation of the driest month (bio 14), precipitation of the wettest quarter (bio 16), precipitation of the driest quarter (bio 17), precipitation of the warmest quarter (bio 18), and precipitation of the coldest quarter (bio 19). Precipitation is correlated with mean maximum temperature ($r = -0.135$, $p < 0.05$). NDVI is correlated with elevation ($r = 0.316$, $p < 0.05$) and precipitation ($r = -0.0396$, $p = < 0.05$). For variable pairs with P values greater than 0.050 there is no significant relationship between the two variables.

In summary, in the Hampton Roads region there is a significant inverse correlation between mosquito collection data and both NDVI and elevation data. Mosquito populations tend to decrease with both increasing NDVI and elevation. Converse to the Northern Virginia and Richmond-Petersburg regions, NDVI values were lower at lower elevations. There is a direct relationship between mosquito collection data and precipitation

data. Mosquito populations tend to increase with increasing precipitation. There is no significant correlation between mosquito collection data and temperature data, however, there is a significant positive correlation between bioclimatic variables that captured the affects of both temperature and precipitation. NDVI is greater at higher elevations and when precipitation is lower. Mean maximum temperature is lower when precipitation is greater.

Table 4.9. Correlation between mosquito density and environmental-climatic predictor variables. The Pearson correlation coefficient (R) and P-value (in parentheses) are shown.

Predictor Variable	***Northern Virginia (n = 1443) Correlation Coefficient (P-value)***		***Richmond-Petersburg (n = 773) Correlation Coefficient (P-value)***		***Hampton Roads (n = 2715) Correlation Coefficient (P-value)***	
Elevation	***-0.0473	(0.0722)	**-0.1030	(0.0043)	-0.1070	(0.0000)
Monthly mean maximum temperature	*0.0851	(0.0012)	-0.0692	(0.0544)	0.0302	(0.1290)
Monthly mean minimum temperature	0.0842	(0.0014)	-0.0573	(0.1120)	0.1760	(0.0000)
Monthly mean precipitation	-0.0237	(0.3690)	-0.0290	(0.4210)	0.0479	(0.0161)
Monthly mean maximum NDVI	0.1410	(0.0000)	-0.0682	(0.0581)	-0.1900	(0.0000)
Long-term mean monthly maximum temperature	0.0873	(0.0009)	-0.0953	(0.0080)	0.0273	(0.1700)
Long-term mean monthly minimum temperature	0.0865	(0.0010)	-0.0923	(0.0103)	0.0692	(0.0005)
Long-term mean monthly mean precipitation	0.0464	(0.0781)	-0.0516	(0.1520)	-0.0353	(0.0761)
Long-term mean monthly maximum NDVI	0.1320	(0.0000)	-0.1850	(0.0000)	-0.2120	(0.0000)

Predictor Variable	*Northern Virginia (n = 1443) Correlation Coefficient (P-value)*		*Richmond-Petersburg (n = 773) Correlation Coefficient (P-value)*		*Hampton Roads (n = 2715) Correlation Coefficient (P-value)*	
Bio 01	0.0699	(0.0079)	0.1450	(0.0001)	0.0390	(0.0403)
Bio 02	-0.0174	(0.5100)	-0.0127	(0.7250)	-0.2650	(0.0000)
Bio 03	0.0432	(0.1010)	0.0570	(0.1130)	-0.2740	(0.0000)
Bio 04	-0.1550	(0.0000)	-0.0237	(0.5100)	0.0741	(0.0002)
Bio 05	-0.0207	(0.4320)	0.1380	(0.0001)	-0.0787	(0.0000)
Bio 06	0.0639	(0.0152)	0.1230	(0.0006)	0.1760	(0.0000)
Bio 07	-0.0813	(0.00201)	-0.0275	(0.4450)	-0.1860	(0.0000)
Bio 08	0.0092	(0.7280)	-0.0737	(0.0404)	0.3110	(0.0000)
Bio 09	0.0806	(0.0022)	0.0565	(0.1170)	0.0908	(0.0000)
Bio 10	0.0378	(0.1510)	0.1320	(0.0002)	0.0428	(0.0315)
Bio 11	0.0802	(0.0023)	0.1500	(0.0001)	0.0527	(0.0081)
Bio 12	-0.0594	(0.0239)	0.0518	(0.1500)	-0.1410	(0.0000)
Bio 13	0.0445	(0.0911)	0.1020	(0.0047)	-0.1190	(0.0000)
Bio 14	0.0613	(0.0198)	0.0105	(0.7700)	-0.2420	(0.0000)
Bio 15	-0.0696	(0.0082)	0.1120	(0.0018)	0.0420	(0.0348)
Bio 16	-0.0336	(0.2020)	0.1230	(0.0006)	-0.1840	(0.0000)
Bio 17	0.0510	(0.0528)	0.0658	(0.0676)	-0.2800	(0.0000)
Bio 18	-0.0492	(0.0617)	0.1280	(0.0003)	-0.2480	(0.0000)
Bio 19	0.0510	(0.0528)	0.0886	(0.0137)	-0.3000	(0.0000)

* The pair(s) of variables with positive correlation coefficients and P values below 0.050 (shown here in red) tend to increase together.
** For the pairs with negative correlation coefficients and P values below 0.050 (shown here in blue), one variable tends to decrease while the other increases.
***For pairs with P values greater than 0.050 (shown here in black), there is no significant relationship between the two variables.

4.4 COMPETENT VECTOR PREDICTED DISTRIBUTION

Initially, all environmental and climate variables were included in MaxEnt model development. Jackknife tests of variable importance revealed that land-cover was the most influential variable in model development; the training gain when land-cover was the only variable used in model development was high, indicating that it contributed strongly to the model and that the land-cover variable contains unique information that is required for model creation. Points classified as "urban and built-up" coverage were associated with high probabilities of presence. These areas correspond to the location of the majority of the training and test locations; most of the mosquito surveillance data for the state was collected in urban areas. Although the distribution of mosquitoes is certainly, in part, related to land-cover (e.g., the presence or absence of wetlands, the type of surrounding vegetation), land-cover was eliminated from further analyses because of sampling bias.

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The MaxEnt cumulative predictive model for the suitability of RVF competent vector habitat in Virginia is shown in Figure 4.11. The reliability of the model was assessed by the Area Under the Curve (AUC) procedure. The AUC for the training points was 0.982 and for the test points was 0.973 with a standard deviation of 0.003, which indicates the model had a good capacity for distribution prediction.

The minimum predicted value assigned to training localities (0.012) was used to set a threshold for converting continuous values to binary ones to create a presence-absence competent vector distribution. Suitability values <0.012 were reclassified as 0 (absence) and suitability values >0.012 were reclassified as 1 (presence). The presence-absence MaxEnt model for RVF competent vectors in Virginia is shown in Figure 4.12. The fractional predicted area (the area coded as 1 = present) is 0.465, and the omission rate for test points

was 0.003. At this threshold, the test points were classified significantly better by the model than would be expected from random ($p<0.0001$).

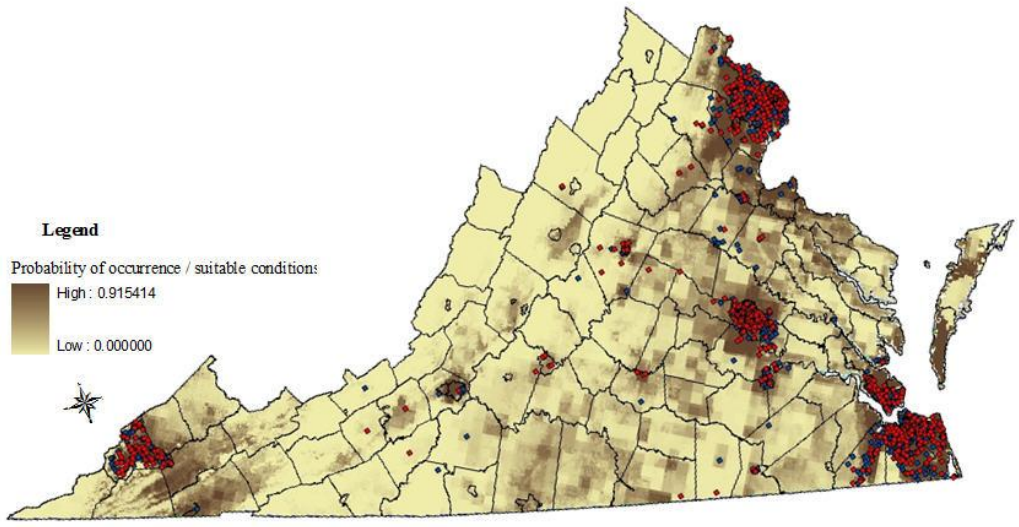

Figure 4.11. Predicted distribution of RVF competent vector species in Virginia. The distribution map was created by importing the MaxEnt cumulative output into ArcGIS 9.2. Darker areas indicate high probability of suitable conditions for or occurrence of the RVF competent vectors in Virginia; light areas indicate low predicted probability of suitable conditions or species occurrence. Red dots show the presence locations used for training while blue dots show test locations.

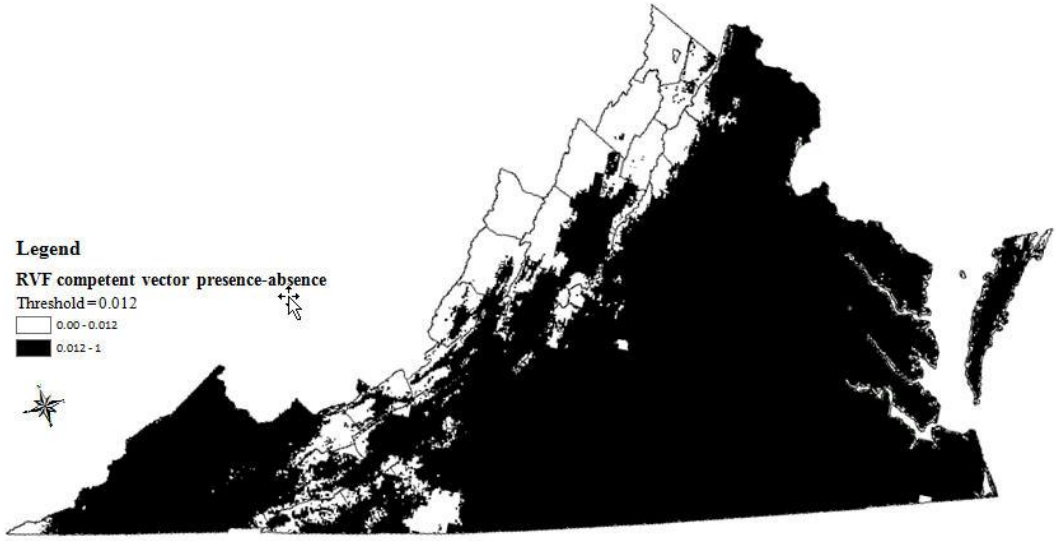

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Figure 4.12. Presence-absence predicted distribution of RVF competent vector species in Virginia. The distribution map was created by importing the MaxEnt cumulative output into ArcGIS 9.2. Light areas indicate low probability of species occurrence and dark areas indicate high probability of species occurrence.

The Jackknife test of variable importance (Figure 4.13) revealed the environmental variable with highest gain when used in isolation is long-term maximum NDVI (max_n_a_allc), which by itself appears to have the most useful information. Minimum temperature (min_tminc), minimum temperature in the coldest month (bio_06c), and mean temperature of the driest quarter (bio_09c) had the next greatest contribution to the model when used in isolation. When elevation (va_altc) is omitted, gain is decreased the most. This indicates that elevation has the most information useful for model development that is not present in other environmental-climatic variables. When elevation and temperature are excluded from model development, the environmental variable that decreases the gain the most when it is omitted is mean precipitation (mean_vaprecpc).

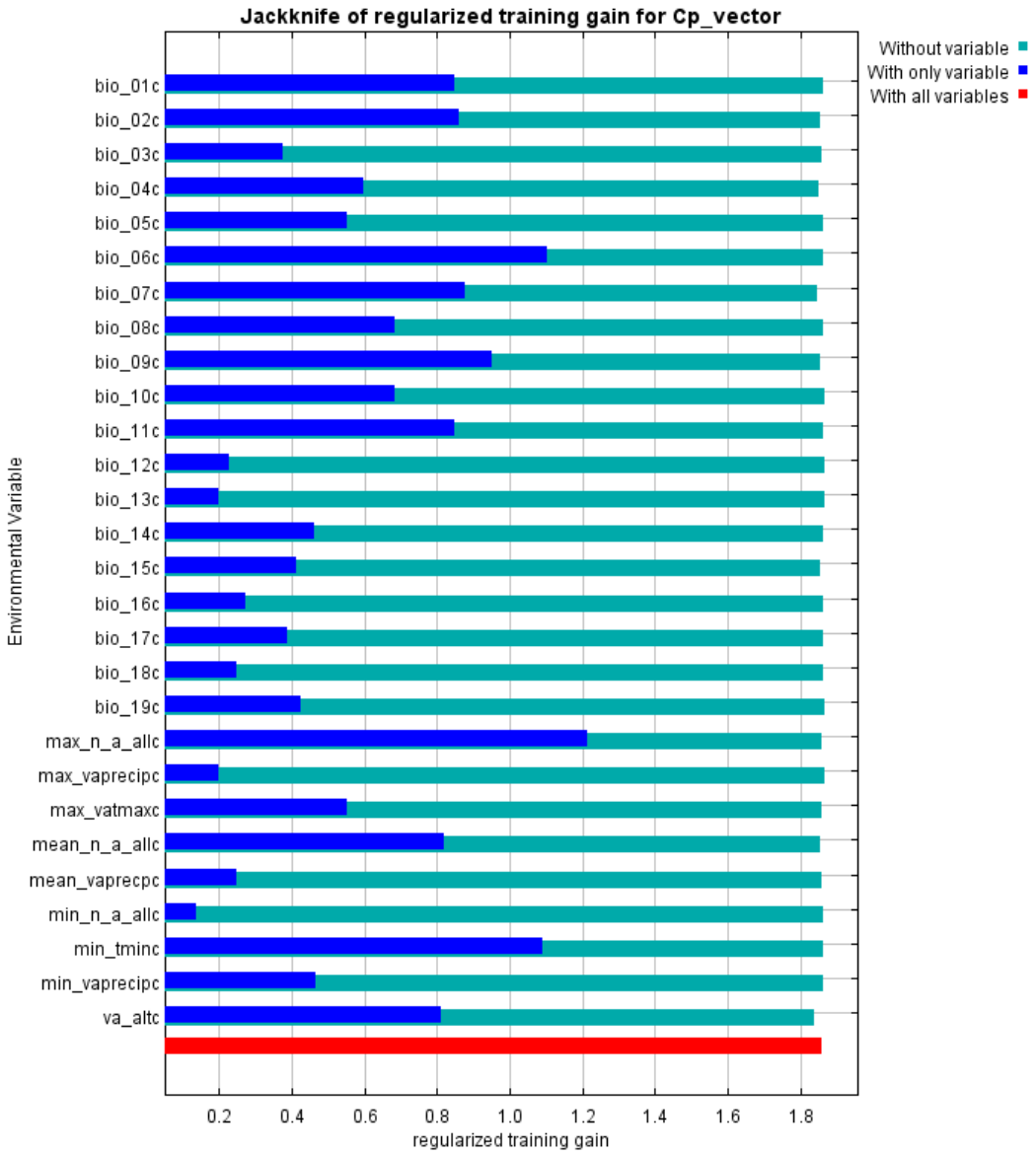

Figure 4.13. Jackknife test of training gain for RVF competent vectors. The environmental predictor variables used in the MaxEnt species distribution model included the Worldclim bioclimatic variables (Table 3.4) represented by bio_01c through bio_19c; minimum, mean and maximum NDVI represented by min_n_a_allc, mean_n_a_allc, and max_n_a_allc respectively; minimum and maximum temperature represented by min_tminc and max_vatmaxc; minimum, mean, and maximum precipitation represented by min_vaprecipc, mean_vaprecpc, and max_vaprecipc; and SRTM elevation represented by va_altc.

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4.5 Risk of RVF Establishment and Transmission to Humans in Virginia

The weighted suitability modeling process to create a risk map for the establishment of RVF in Virginia is shown in Figure 4.14. Domestic animals (cattle, sheep, and goats) and white-tailed deer were considered to be the principal hosts that would play a role in the establishment of the RVF virus in Virginia. Domestic animals were assigned a weight of 0.50 due to their established importance with respect to the ecology and epidemiology of the virus in RVF endemic regions of the world and relative importance in U.S. agricultural economy: out of the 1,119,517 farms that raised 101,772,203 domestic ruminants in the U.S. in 2002, Virginia is one of 12 states that together had 32.37% and 31.85% of all the farms and animals, respectively (USDA 2006). White-tailed deer were also assigned a weight of 0.50 due to the high density and widespread distribution of the species among areas of high human population (VDGIF 2009), and the potential importance the RVF virus naïve population may play in potential RVF virus establishment in Virginia. The resulting interim Host layer and Vector layer (MaxEnt mosquito habitat suitability model) were then assigned equal weights (0.50) to sum to 1.0. The resulting risk map for the establishment of RVF in Virginia is displayed in Figure 4.15. For descriptive purposes, outputs of the weighted suitability model were classified using histograms to indicate very low and low risk in green, medium risk in yellow, and high and very high risk in the red.

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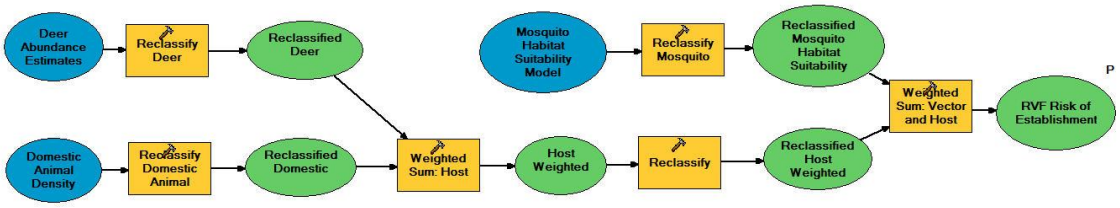

Figure 4.14. Chart from ArcGIS ModelBuilder application showing construction of weighted sum model for the risk of RVF virus establishment. In this model, the weighted sum process was first run to product a "Host" layer. Weights assigned to host species were: Domestic animals, 0.50 and Deer, 0.50. The Host and Vector (MaxEnt Mosquito Habitat Suitability model) layers were reclassified and equally weighted, and the weighted sum process was run again to produce a risk map.

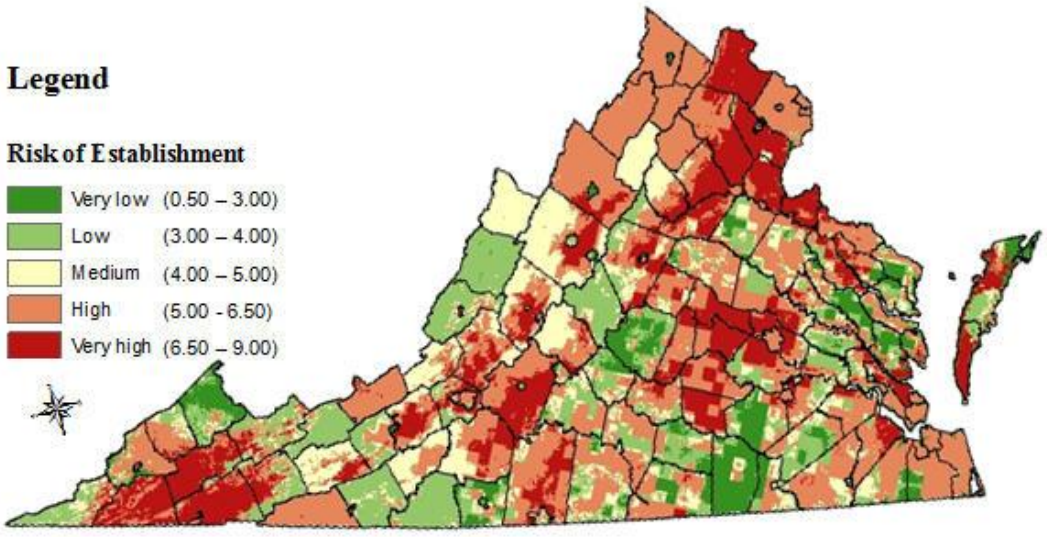

Figure 4.15. Risk map for RVF virus establishment in Virginia. The risk ranges from 0.50 – 9.00, with a mean risk of 5.06.

The weighted suitability modeling process to create a risk map for the transmission of RVF virus to humans in Virginia is shown in Figure 4.16. The risk of RVF virus transmission to humans was determined by assigning the risk of RVF virus establishment model and human density layer equal weights (0.50) to sum to 1.0. The weighted sum process was again run with the risk of establishment and human density layers to create the overall risk of RVF virus transmission to humans map (Figure 4.17). Again, for descriptive purposes, outputs of the weighted suitability model were classified using histograms to indicate very low and low risk in green, medium risk in yellow, and high and very high risk in the red.

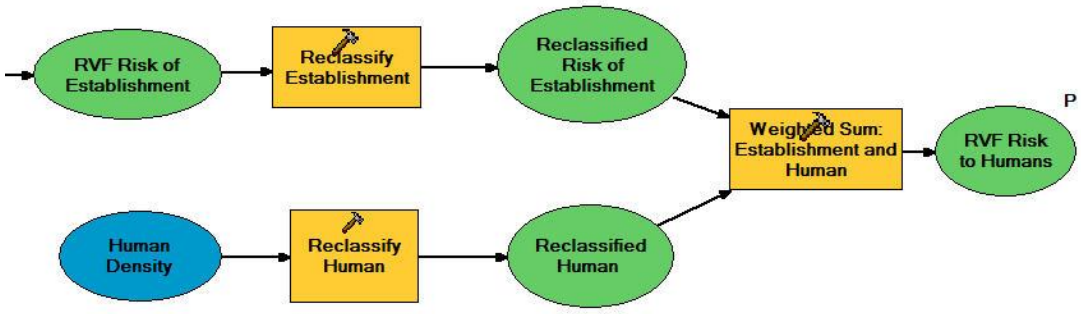

Figure 4.16. Chart from ArcGIS ModelBuilder application showing construction of a weighted sum model for risk of RVF virus transmission to humans. The Human Density layer was added to the RVF risk of establishment model, the layers were reclassified and equally weighted, and the weighted sum process was run again to produce a risk map.

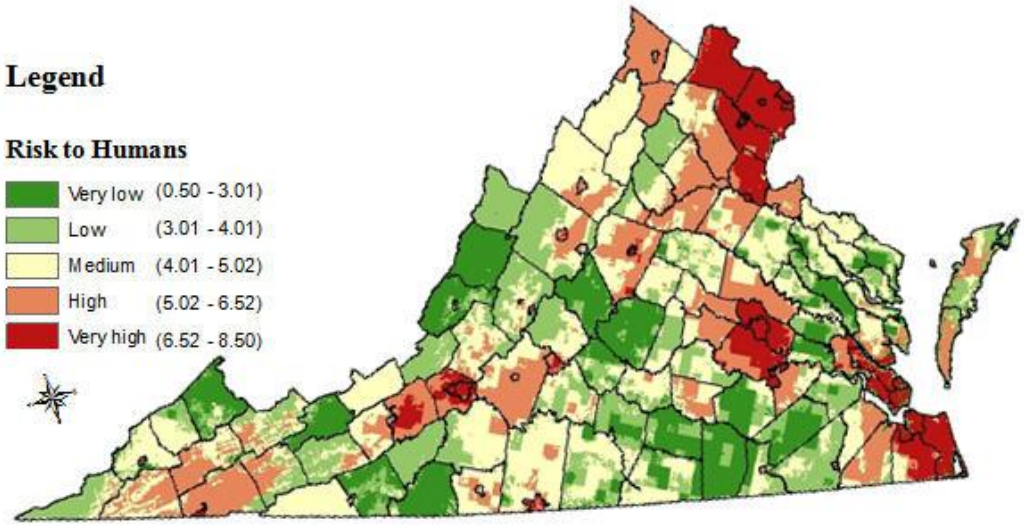

Figure 4.17. Risk map for RVF virus transmission to humans in Virginia. The risk ranges from 0.50 – 8.50, with a mean risk of 4.62.

CHAPTER 5. DISCUSSION

5.1 ENVIRONMENTAL-CLIMATIC VARIABLES AS PREDICTORS OF MOSQUITO DENSITY

Pearson's Product Moment Correlation Coefficient analyses addressed mosquito density spatial and temporal patterns through the use of remote sensing data. Significant correlations between mosquito and environmental-climatic data affirmed that predicting the spatial and temporal dynamics of disease vector species is feasible. Although there were variations in climate-population relationships among the regions, Normalized Difference Vegetation Index (NDVI), elevation, temperature, and precipitation all appear to be important predictors of mosquito abundance and distribution in Virginia.

Overall, it was affirmed that mosquito populations can be influenced by variations in climate, as measured by Normalized Difference Vegetation Index (NDVI). NDVI is a proxy variable of local conditions (vegetation and water) that affect the development of mosquitoes. Although, mosquito abundance and distribution in Virginia is directly correlated with NDVI, the relationship varied among regions. In Northern Virginia, mosquito populations tend to increase with increasing NDVI.

Conversely, in the Hampton Roads region mosquito population density was greater where NDVI values were lower. The inverse relationship between mosquito population and NDVI in the Hampton Roads region may partly be a function of land-cover. Most of the

mosquito data from this region was collected in urban areas. This was also true of the Northern Virginia region, but the NDVI values in urban areas of Northern Virginia (e.g., >0.6 in June 2005) were generally higher than those for Hampton Roads (e.g., < 0.5 in June 2005) and closer to NDVI values in surrounding areas.

The inverse relationship between mosquito population and NDVI in the Hampton Roads region may also be a function of elevation and associated vegetation. Elevation influences factors, including temperature, rainfall and humidity (Guerra *et al.* 2006), that affect vector and animal distributions as well as vegetation. Low elevations may be more prone to accumulation of ground surface water and flooding, producing habitats suitable for mosquitoes (Clements *et al.* 2006). Elevation is significantly correlated with mosquito density in the Hampton Roads region, where mosquito populations tend to decrease with increasing elevation. The majority of the mosquito data from Hampton Roads was collected in flat, low-relief regions along major rivers and near the Chesapeake Bay or low, open areas covered with sediment and vegetation in direct proximity to the Chesapeake Bay and Atlantic Ocean (e.g., barrier islands and salt marshes). In this region water accumulates in ditches and puddles after rains. The predominant species collected here, *Cx. salinarius*, can develop in the freshwater habitats with dying vegetation, in ditches and puddles where water accumulates after rains, as well as in brackish conditions found near salt marshes (Crans 2004). In these areas, mosquito populations are high, however, NDVI values are lower possibly due to the type of vegetation (grasses) and the amount of water within and surrounding the vegetation.

The Northern Virginia and Richmond-Petersburg regions area are mostly hilly, and the majority of the species collected in those areas are container breeders which do not rely as

heavily on rainfall as do the predominant floodwater mosquito species found in the Hampton Roads region.

Although there is not a significant correlation between mosquito density and NDVI in the Richmond-Petersburg region, there is a significant inverse relationship between NDVI and elevation as well as mosquito density and elevation. That is, mosquito populations tend to be greater at lower elevations where NDVI values are higher.

In the present study, temperature has a moderately strong direct relationship with mosquito abundance. This association is in agreement with other studies that have demonstrated similar relationships in mosquito species. One of the key elements for mosquito development and survival is temperature. Temperature affects the length of time the mosquito larvae develop (Moore and Sutherland 1986), host seeking behaviors, egg laying, and the length of time the mosquito larvae develop (Moore and Sutherland 1986).

Vectors have optimal temperature ranges. Higher temperatures increase host seeking and are associated with greater egg laying, but temperatures that are too high become unfavorable for mosquito survival (Moore and Sutherland 1986, Shone *et al.* 2006). Patz *et al.* (1996) concluded that mosquitoes do not usually survive where the mean winter temperature drops below 16-18 degrees Celsius. Excessively high temperatures shortened vector life-spans (Craig *et al.* 1999, Clements *et al.* 2006). Intermediate temperatures are assumed to be most conducive to stable vector populations.

Although, mosquito densities in Virginia are directly correlated with temperature, as with NDVI, the relationship varied among regions. In the Northern Virginia region, the density of mosquitoes collected is positively associated with temperature. Conversely, mosquito populations in the Richmond-Petersburg and Hampton Roads regions increase with

decreasing temperature. The inverse relationship between mosquito density and temperature could be a reflection of the correlation of temperature and precipitation (Alto and Juliano 2001). In the Richmond-Petersburg and Hampton Roads regions, temperature is inversely associated with precipitation. It appears that precipitation events may induce a reduction in temperature while increasing mosquito densities in these regions.

In terms of mosquito densities throughout Virginia, low precipitation may be non-conducive to maintenance of stable vector populations, while extremely high precipitation may limit the presence of stable habitats for the developmental stages of the vector populations. As with temperature, intermediate amounts of precipitation is assumed to be most conducive to stable vector populations.

It is important to note that the data included in these analyses is for only two years (2005-2006). Only 2005-2006 mosquito collections were selected because the sampling efforts in other years (2000-2004) were not as extensive. This particular issue may affect the ability of this study to detect relationships between mosquito populations and environmental-climatic variables.

Nevertheless, the results of these analyses are promising. Mosquito density spatial and temporal patterns were explored through the use of remote sensing data and correlations between mosquito and environmental-climatic data were detected, confirming feasibility of using environmental-climatic data to predict the spatial and temporal dynamics of disease vector species. In particular, a mosquito density-NDVI relationship across space (as detected in correlation analyses) and across time (as observed in graphical analyses) is evident, and this forms the basis for using NDVI to develop a predictive model for un-sampled areas in Virginia.

5.2 Environmental-Climatic Variables as Predictors of Mosquito Distribution

The MaxEnt species distribution prediction model presented in this study is robust, having a high accuracy assessment by the Area Under the Curve (AUC) measurement. The resulting species distribution map shows the estimated distribution of potential RVF competent vectors in Virginia, as a function of the environmental and climatic variables included in the study.

Most of Virginia was estimated to have habitat suitable for the presence or occurrence of potential RVF competent vectors. The highest probability of occurrence was generally along the eastern portion of the state and coincided with urban areas and/or coastal areas at lower elevation with relatively warmer temperatures.

The suitability for occurrence of competent vectors when multiple factors are present was shown to be greater than the suitability associated with the individual factors. NDVI, a proven biologically realistic index of environmental factors influencing mosquito populations, was the most useful variable in model development. Since animal life depends on vegetation, tracking spatial-temporal variation in vegetation similarly evaluates animal populations (Hielkema *et al.* 1986). However, using NDVI alone in model development did not result in as robust a model as including other environmental-climatic factors with NDVI. Elevation and temperature were also important, although these variables did not contribute to model development as much as NDVI.

The low contribution of precipitation to model development may be the result of the variable being correlated with others (e.g., temperature, as shown in this study with Pearson's Product-Moment Correlation Coefficient analyses). Since environmental-climatic variables may be correlated (e.g., lower elevations have warmer temperatures), a particular factor may

be important to mosquito biology but may not be an important contribution to the model because another correlated layer is used in the model. Precipitation significantly contributed to model development when both elevation and temperature were removed.

All the bioclimatic variables contributed toward model development, however, those bioclimatic variables derived from both temperature and precipitations generally were not useful in isolation. This is another example of how relative contributions of each variable can be affected by how correlated environmental-climatic variables are to each other. The low contribution of these bioclimatic variables is a reflection of the correlated nature of the temperature and precipitation values from which they were derived. Incidentally, the bioclimatic variables derived solely from temperature values were useful in model development in isolation.

Although land-cover contributed strongly to model development, it was excluded from these analyses. The urban land-cover class was associated with a high probability of presence (unpublished data). The species selected for this study preferentially largely feed on mammals, including humans. The presence of mosquitoes could be more probable in urban environments because humans are more readily available to feed upon. However, the data could be biased due to the fact that mosquito surveillance practices are predominantly accomplished in urban areas.

The complementary use of an ecological niche modeling method like MaxEnt and environmental-climatic habitat suitability estimators resulted in a geographical representation of candidate RVF competent vector distribution in Virginia and a measure of its accuracy. However, a representation produced in this way has biases and limitations. It is important to recognize that sampling bias likely affected the species prediction map presented in this

study; predicted suitable habitat was largely in locations where mosquito data was collected. Another limitation to distribution modeling is spatial clustering. Surveillance data is often spatially clustered (as in this study) which can reduce the statistical significance of distribution models. A third limitation is spatial autocorrelation, a common characteristic of distribution data (Legendre 1993, Estrada-Pena 1998). Spatial autocorrelation is the tendency for data from sample locations in close proximity to each other to be more similar than data from sites located farther apart (ESRI 2008). Autocorrelated data violate the assumption of independence of most standard statistical procedures (Legendre 1993).

Also noteworthy, the model developed for this study is a prediction of suitable habitat for nine potential competent vector species. Since the measure of mosquito distribution used in this study was based on potential RVF competent vectors as a group, there is no way to determine whether individual species might have had slightly different responses to the environmental-climatic variables. In addition, the actual distribution ranges of the species can be affected by other factors such as interactions with other species and their distribution may change over time with changing environmental-climatic factors.

5.3 Risk Maps

Two risk maps were created in this study: the risk of RVF establishment and the risk of RVF transmission to humans. As evident with both risk maps, the probability or risk when multiple factors are present is greater than the probability or risk associated with the individual factors. Areas at medium-to-high risk are located where areas with a higher probability of competent vector occurrence overlap with densely-populated host areas. In short, there is greater opportunity for host-vector interactions and virus transmission where

denser populations of hosts occur in habitats suitable for vectors. The distribution of high risk areas throughout the state could result in rapid statewide spread of the virus if it is introduced in just one of these areas.

In the previous chapter, risk of establishment of RVF virus across Virginia was visually represented in Figure 4.15. Based on the epidemiology of RVF and ecology of RVF virus, domestic animals (cattle, sheep and goats) and wildlife (white-tailed deer) were included as the principal host groups involved in the initial phase of an outbreak and consequential virus establishment in local mosquito populations. Areas at medium-to-high risk of RVF establishment in mosquito populations are located where areas with a higher probability of competent vector occurrence overlap with densely-populated domestic animal and/or white-tailed deer areas. In general, the risk of establishment is greatest in the Northern Virginia, Richmond-Petersburg, and south-western (e.g, Roanoke City/County, Montgomery County, and Washington County) regions of Virginia.

Estimating the probability of establishment within vectors is an integral part of estimating the probability of infection to humans. Consequently, human density was evaluated with respect to risk of RVF establishment to estimate the risk of RVF transmission to humans (Figure 4.17). As expected, risk of RVF transmission to humans is greatest in areas of the state with the largest human populations, namely the Northern Virginia, Richmond-Petersburg, and Hampton Roads areas. High risk in the Northern Virginia, Richmond-Petersburg, and Hampton Roads regions is associated with high probabilities of competent vector occurrence, high white-tailed deer population estimates, and dense human populations. The south-west part of the state (e.g, Roanoke City/County, Montgomery County, and Washington County), in general, is more rural than the eastern portion of the

state. The high risk in this region is more indicative of a combination of all factors: domestic animal, white-tailed deer, human, and vector occurrence. Although dense human and domestic animal populations by and large do not coincide throughout Virginia, in the south-west part of the state there high risk areas with pockets of medium-to-high human density near both livestock production and dense populations of deer.

A major issue with the methods applied in this study is subjectivity, particularly with regards to defining the weights of the vector and host species included in risk map development. Notably, changing the models and the relative weights of the species categories primarily affected the extent and level of the risk, but the overall pattern of risk was similar (unpublished data). For example, weighting the mosquito layer more heavily produced risk of establishment and risk of transmission maps that more closely resembled the MaxEnt mosquito habitat suitability model; risk areas on the coast (e.g., Hampton Roads and Northern Virginia regions) where mosquitoes are more probable were highlighted. As another example, weighting domestic animals more heavily resulted in risk maps that highlighted western portions of the state where livestock production is more prevalent.

CHAPTER 6. CONCLUSIONS AND FUTURE DIRECTIONS

6.1 SUMMARY OF GOALS AND KEY FINDINGS

The primary goal in this study was to explore remote sensing, ecological niche modeling, and geographic information systems as aids in predicting candidate RVF competent vector abundance and distribution in Virginia, and as means of estimating where risk of establishment in mosquitoes and risk of transmission to human populations would be greatest in Virginia. As part of this study, a mosquito surveillance database was compiled to archive the historical patterns of mosquito species abundance in Virginia. In addition, the spatial and temporal relationship between mosquito activity and local environmental and climatic patterns were examined.

The present study affirms the potential role of remote sensing imagery for species distribution prediction, and it demonstrates that ecological niche modeling is a valuable predictive tool to analyze the distributions of populations. The MaxEnt ecological niche modeling method used here successfully predicted habitat suitability for RVF mosquito vectors in Virginia as a function of the environmental-climatic factors that affect mosquito bioecology or activity. The resulting species distribution map estimates areas with suitable environmental conditions to support potential RVF competent mosquito populations. Most of Virginia was determined to have habitats suitable for mosquito species that may have the capacity to carry and transmit the RVF virus.

The results of this study indicate that the Normalized Difference Vegetation Index (NDVI) – which integrates the combined effects of precipitation, temperature, humidity, elevation, and soils (Linthicum *et al*. 2007) – can be used as a proxy variable of local conditions for the development of mosquitoes to predict mosquito species distribution and abundance in Virginia. However, these results indicate that a more robust prediction can be obtained by including other environmental-climatic factors that affect mosquito densities (e.g., temperature, precipitation, elevation) with NDVI.

Remote sensing and GIS were successful used with ecological niche and risk modeling methods to provide an estimate of the risk of RVF virus establishment in mosquitoes and transmission to humans. The two risk maps presented in this study provide a baseline assessment of Virginia's vulnerability to RVF. One risk map visually represents variation in risk of establishment of RVF virus in potential competent vector mosquito populations across Virginia in the absence of factors that could prevent disease establishment in the area (e.g., public health interventions or mosquito control). The other risk map provides a relative indication of where humans are at risk of contracting the disease based on potential interaction with RVF infected mosquitoes and livestock and/or wildlife. In short, RVF virus establishment in mosquitoes and transmission to humans is likely to occur where competent mosquito vectors geographically overlap with host populations.

It is important to emphasize that the risk map does not reflect the distribution of disease, but it could provide a better understanding of the potential distribution/risk of RVF in the state of Virginia if the disease is introduced to the United States. Also noteworthy, the results of this study give insight into the risk in Virginia of RVF establishment in mosquito populations and transmission to humans, but the risk could be over-reported. This study does

not account for mosquito control efforts, public health interventions or socioeconomic conditions that may affect the true risk. This study also does not account for the fact that risk and effectiveness of control/prevention is dependent on the status of vectors when and where RVF virus introduction may take place. As an example, the risk of establishment would be low if RVF virus introduction occurred in winter; estimates of high risk would be limited to the active mosquito season (April through October, in Virginia). Finally, it is also worth mentioning that the lack of temporally and spatial matched RVF disease incidence data prevents the statistical validation of the accuracy of the risk maps presented in this study. Nevertheless, the strength of this approach to modeling is that it can provide a first order assessment to disease risk when incidence data is lacking.

6.2 Future Directions

Overcome shortcomings of analyses

Shortcomings of the analyses completed in this study are due to unwieldy, incomplete, biased or unavailable data. For instance, although mosquito surveillance data was available for much of Virginia, there was no statewide mosquito surveillance database. At least 80% of time in the study was spent assembling occurrence information and dealing with the challenge of georeferencing the trap sites; this left only a relatively small portion of time to dedicate to analyses. Also noteworthy, biases associated with mosquito surveillance methods (e.g., mosquito surveillance practices are predominantly accomplished in urban areas, trap types are selected based on their ability to collect targeted species) likely affected both spatial analyses in this study.

It is recommended that species diversity and relative species density of mosquito communities be monitored as a basic element of disease surveillance and vector control activities at both a local and statewide level. In addition, the mosquito surveillance data collected on a statewide basis should be available for national analyses. This would enable the analysis of local, regional, and national mosquito distributional patterns and increase knowledge of mosquitoes of medical and veterinary health importance; in turn better mosquito control strategies and large-scale disease preparedness plans could be developed. Finally, stable funding must be provided to develop and maintain viable surveillance programs. Without accurate mosquito density information, mosquito-borne disease risk assessment accuracy may be diminished as risk pertains to mosquito density.

A number of challenges must be overcome to gain a more complete understanding of the risk associated with Rift Valley fever introduction in the United States. Additional vector competency studies are required to increase knowledge about what species in the U.S. could be affected by RVF virus and to estimate the relative importance of these species in a potential outbreak. Further research is also needed on the potential role of vertebrates in the U.S. in propagating the virus and on species-specific responses to the virus.

Data to include in future studies

Some data sets were considered for inclusion in this study, but ultimately were not selected due to unavailability of data (e.g., tire piles) or because they did not fit the scope of this study. For instance, information on human behavior regarding water storage and fine-scale and environmental-climatic conditions that affect mosquito activity (e.g., humidity) are relevant factors with respect to mosquito distribution and mosquito-borne disease transmission, however, they operate on a smaller scale than the other variables included in

this study. This study was intended to generally outline mosquito habitat and potential risk associated with RVF throughout the state of Virginia. The inclusion of local conditions affecting mosquito abundance and distribution in future, smaller scale studies would improve a species distribution model's predictive capabilities.

Inclusion of animal transportation routes and human movement data would not affect the density-based analyses presented in this study. Nevertheless, these factors could affect virus spread, particularly to areas not predicted to be at higher risk. Analyzing the spatial spread of infection requires a detailed map of susceptible host density along with relevant transport links and geographical features. Given the situation where RVF virus has entered the United States, an accurate exposure assessment would have to identify the possible routes by which Virginia livestock and humans would be exposed to the virus (Savill *et al.* 2006).

Fine-scale time series analysis

In this study, a time series analysis was not performed to determine what environmental factors might influence mosquito activity weeks or months in the future. Mosquito population dynamic models incorporating time series analyses using environmental-climatic variables have focused on conditions during or immediately preceding the mosquito breeding season (e.g., Peterson *et al.* 2005) and during the off-season (Walsh *et al.* 2008). The results of these studies indicate that 1) the effects of environmental-climatic conditions are complex, with both direct and indirect mechanisms of action, and probably differ by mosquito species and 2) the activity and geographic distributions of vector species vary in both space and time. In the future, it would be beneficial to examine the time relationships between environmental-climatic variables and mosquito activity. For these analyses, mosquito densities of candidate RVF competent vector species should be examined

individually and over smaller time increments (e.g., 1-day or 10-day counts as opposed to monthly means). The relationships could provide additional, valuable insight to help target and develop mosquito control strategies.

6.3 Applications of this Research

The introduction of RVF virus to the United Sates may be unlikely, however, the geographical range of the disease is widening to involve previously unaffected regions and the current threat of intentional use of biological agents remains. These facts, coupled with the potential deleterious health and economic impacts RVF could have in the United States, make the development of early warning strategies to control the virus spread among mosquitoes and livestock (and potentially wildlife) and to prevent virus transmission to humans of high importance to the United States.

The species distribution and risk maps presented in this study are visual tools by which RVF prevention, detection, and response measures could be determined. These maps identify to mosquito control and public health authorities regions of the state where mosquito control and disease surveillance efforts should be focused in the event that RVF virus is introduced in the United States. With spatially targeted responses, limited resources can be distributed more effectively and the cost of mosquito control and disease surveillance over large areas can be minimized. The results presented here also permit timely, targeted implementation of animal quarantines, dissemination of information, and vaccine strategies to reduce or prevent animal and human disease. The spatial and temporal analyses of mosquito activity included in this study provide public officials information on

environmental conditions that may affect RVF disease reservoirs and the spread of RVF if it is introduced in the United States.

The mosquito surveillance database and methodology of this project is adaptable to the study of any mosquito-borne disease threat. Preparing for one disease by looking at biogeography of its vectors can be informative for many other diseases. For instance, the potential RVF competent vector species studied with respect to RVF are vectors of diseases endemic in Virginia, such as St. Louis encephalitis, Eastern equine encephalitis, and West Nile virus. The information obtained on potential RVF virus vectors and hosts could benefit the study of these and other endemic or emerging mosquito-borne diseases.

6.4 Concluding Remarks

Similar to the introduction of West Nile virus into the United States in 1999, an introduction of RVF into the United States would pose a substantial risk to humans, domestic animals and wildlife populations. As such, preparation for a potential introduction and establishment into the United States is paramount. The present study demonstrates that remote sensing and GIS can be used with ecological niche and risk modeling methods to predict distribution of mosquitoes and risk of disease based on environmental-climatic conditions and mosquito survey data. The resulting candidate RVF competent vector predicted distribution and RVF risk maps presented in this study can help vector control agencies and public health officials focus Rift Valley fever surveillance efforts in geographic areas with large co-located populations of potential RVF competent vectors and human, domestic animal, and wildlife hosts.

APPENDIX A: RVF COMPETENT VECTOR DISTRIBUTION AND ABUNDANCE MAPS

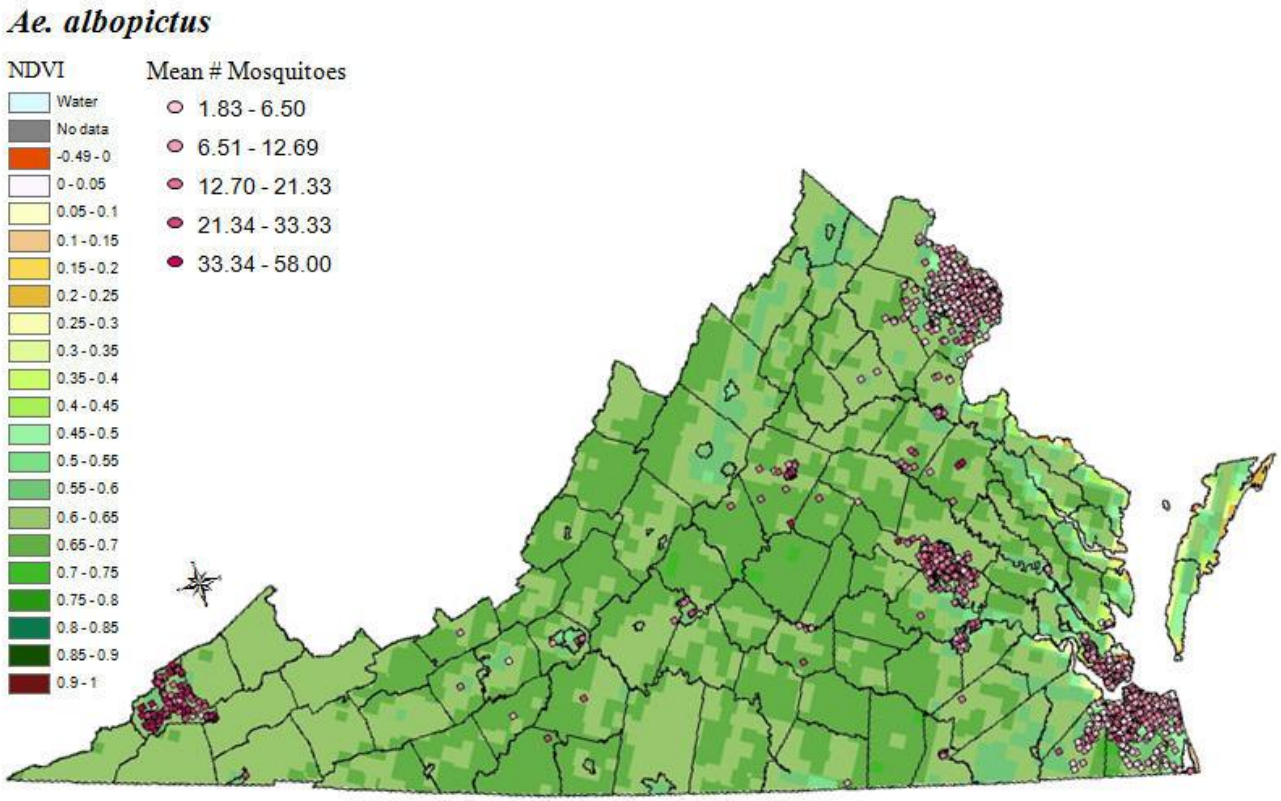

Figure A.1. *Ae. albopictus* distribution and abundance in Virginia.

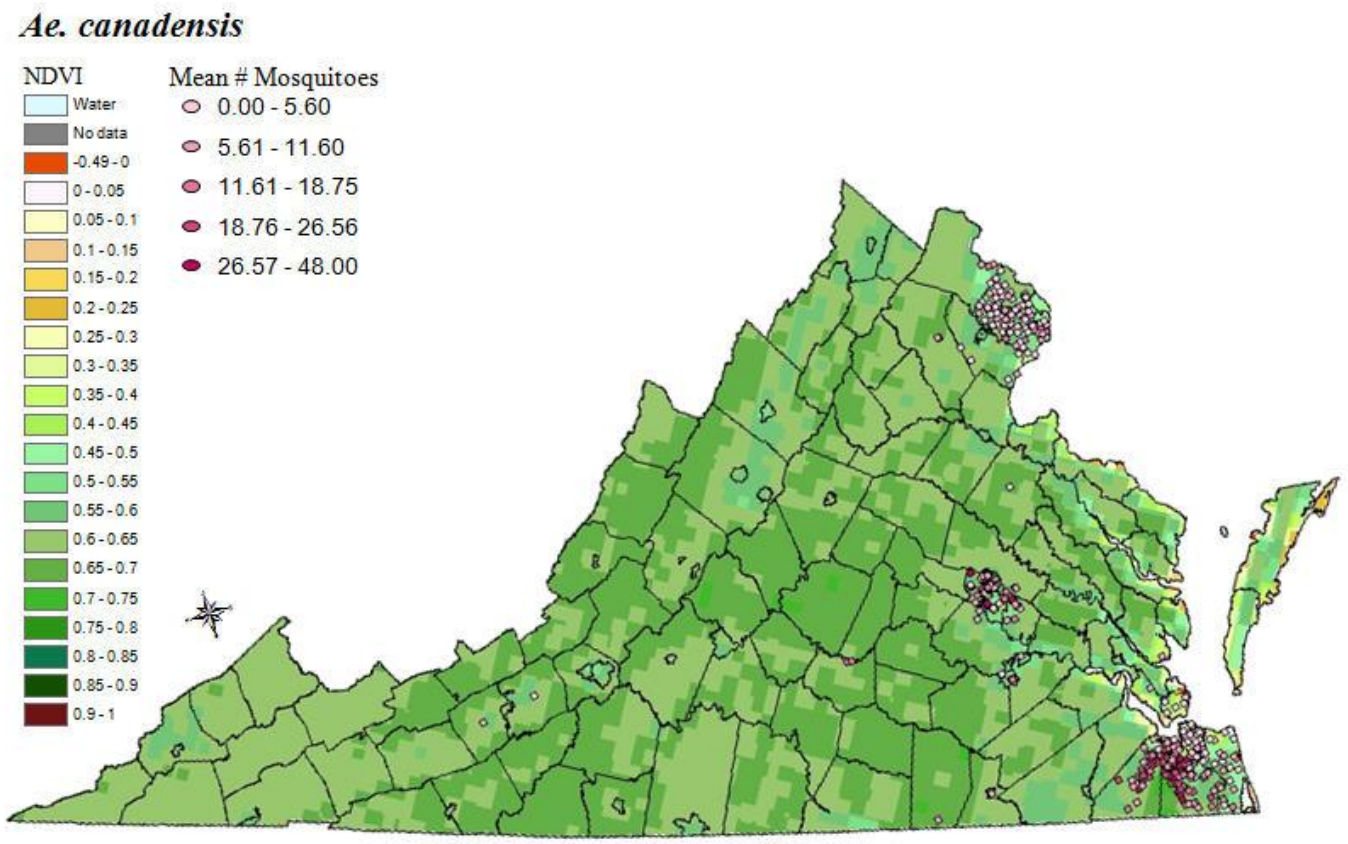

Figure A.2. *Ae. canadensis* distribution and abundance in Virginia.

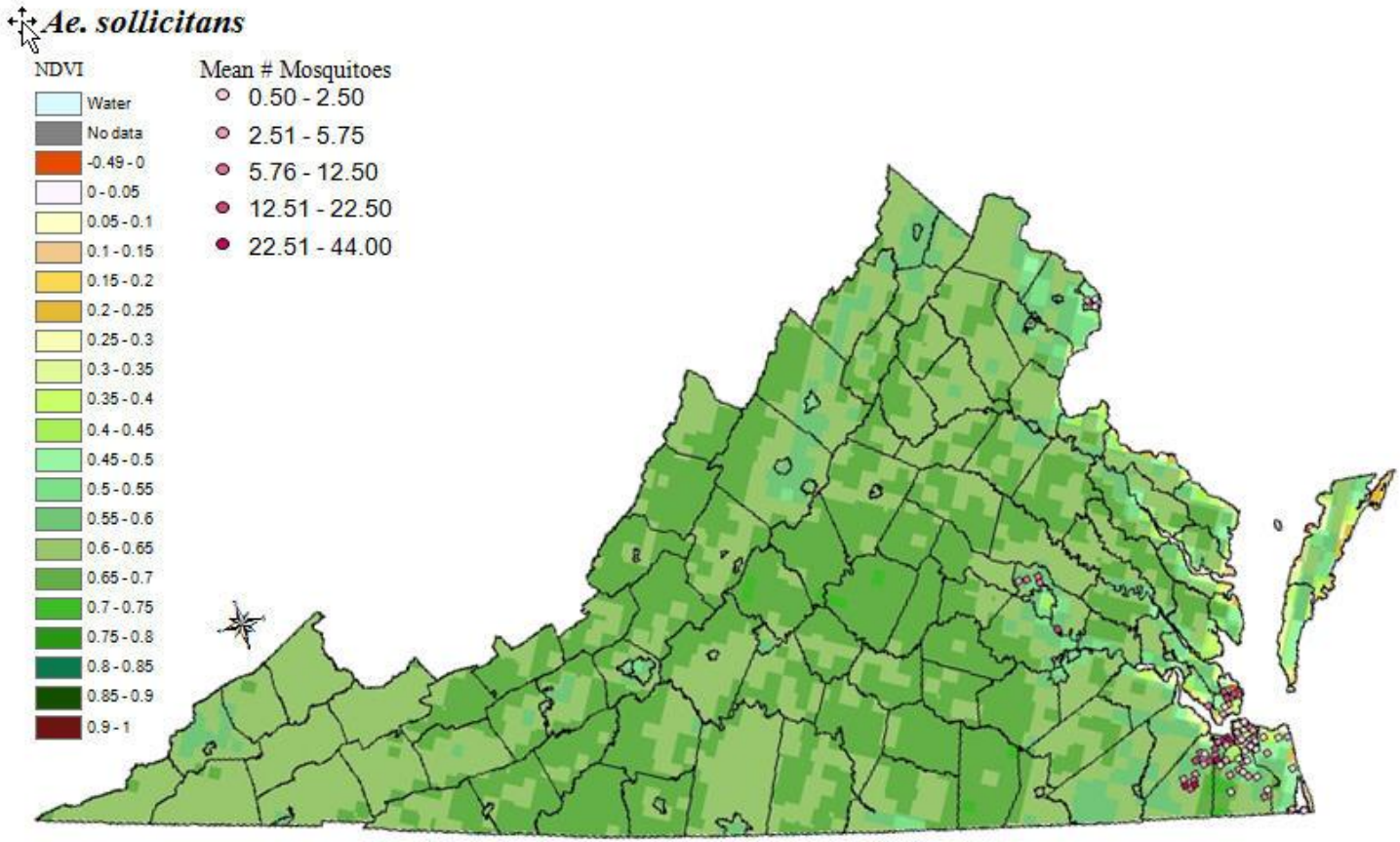

Figure A.3. *Ae. sollicitans* distribution and abundance in Virginia.

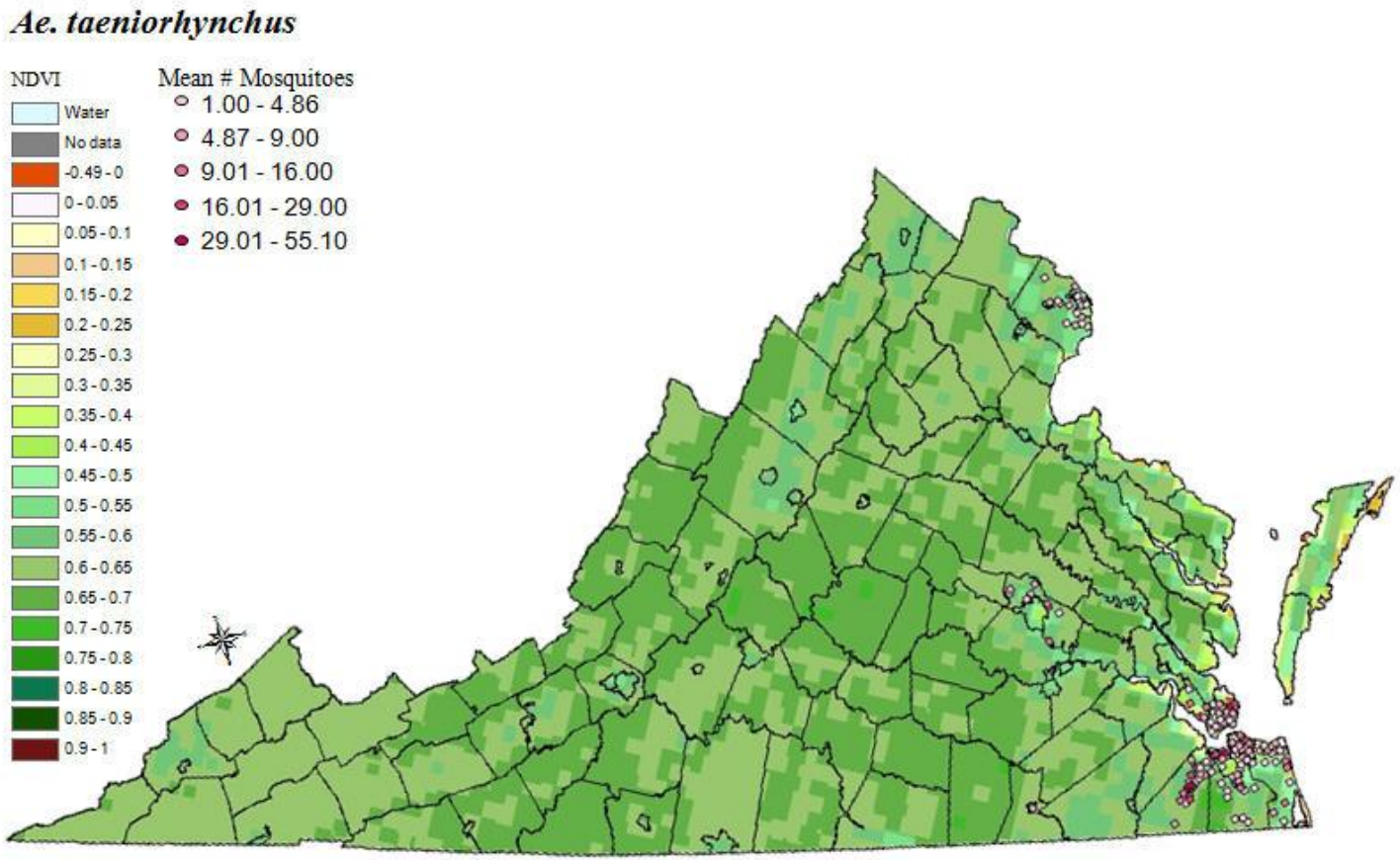

Figure A.4. *Ae. taeniorhynchus* distribution and abundance in Virginia.

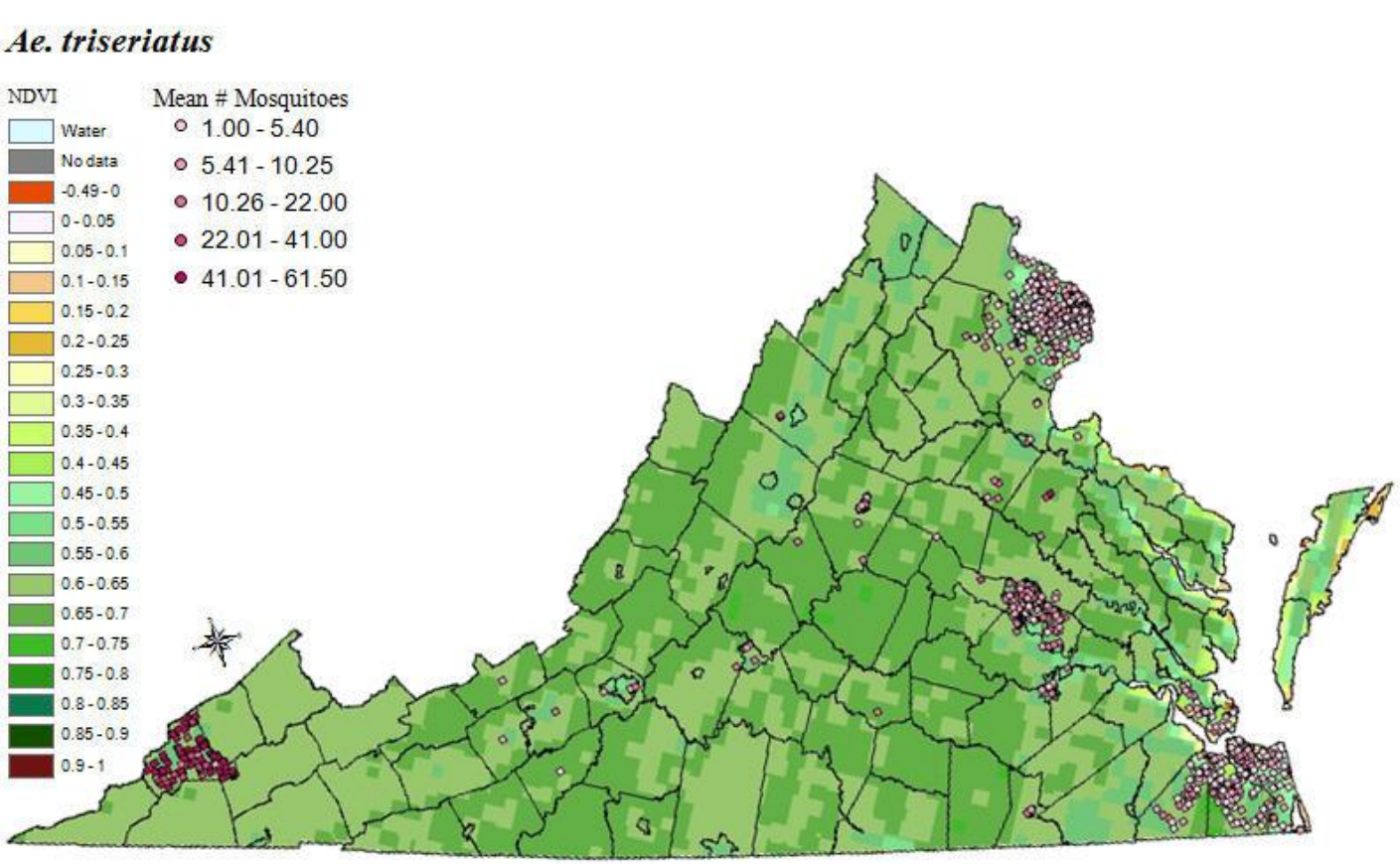

Figure A.5. *Ae. triseriatus* distribution and abundance in Virginia.

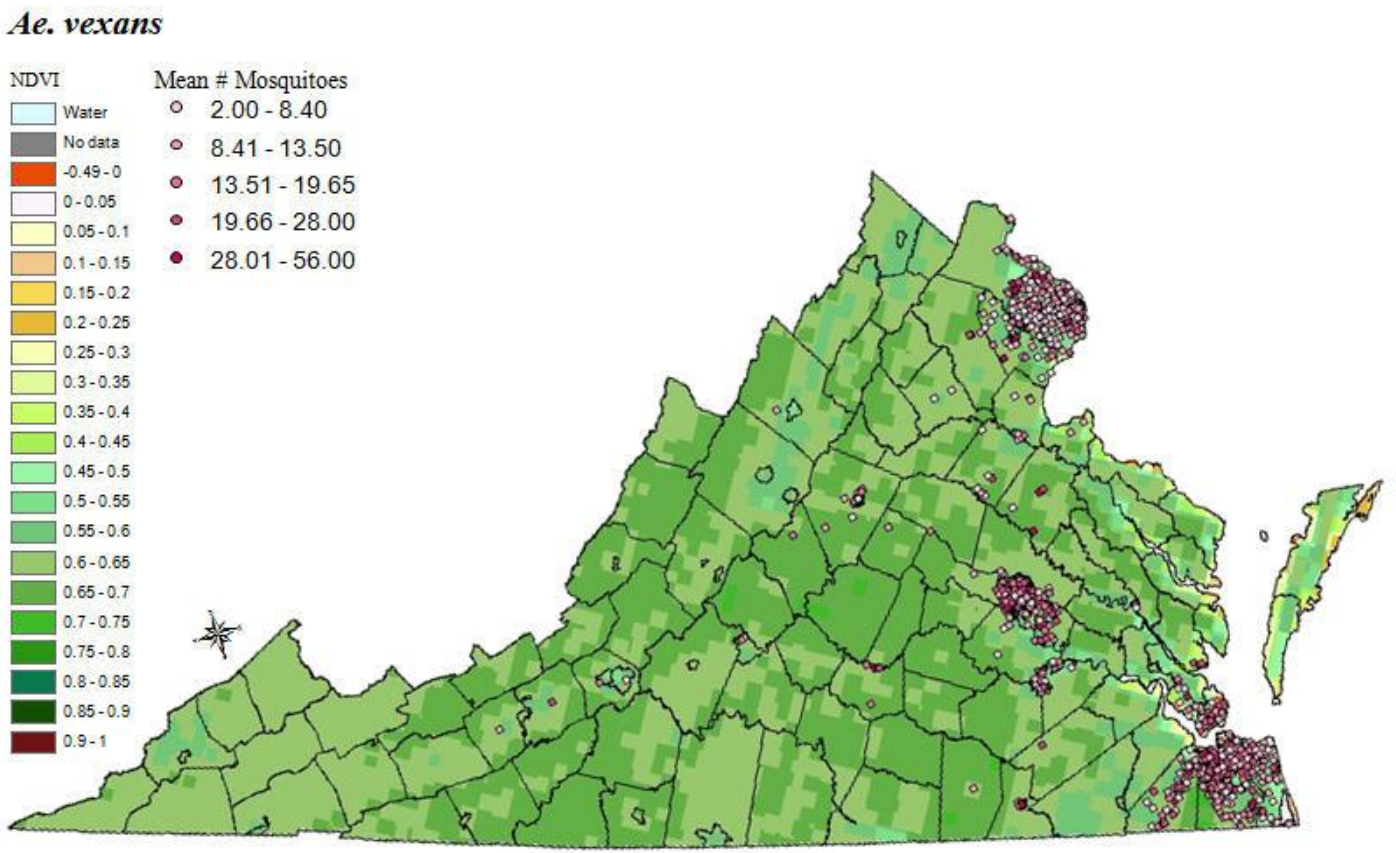

Figure A.6. *Ae. vexans* distribution and abundance in Virginia.

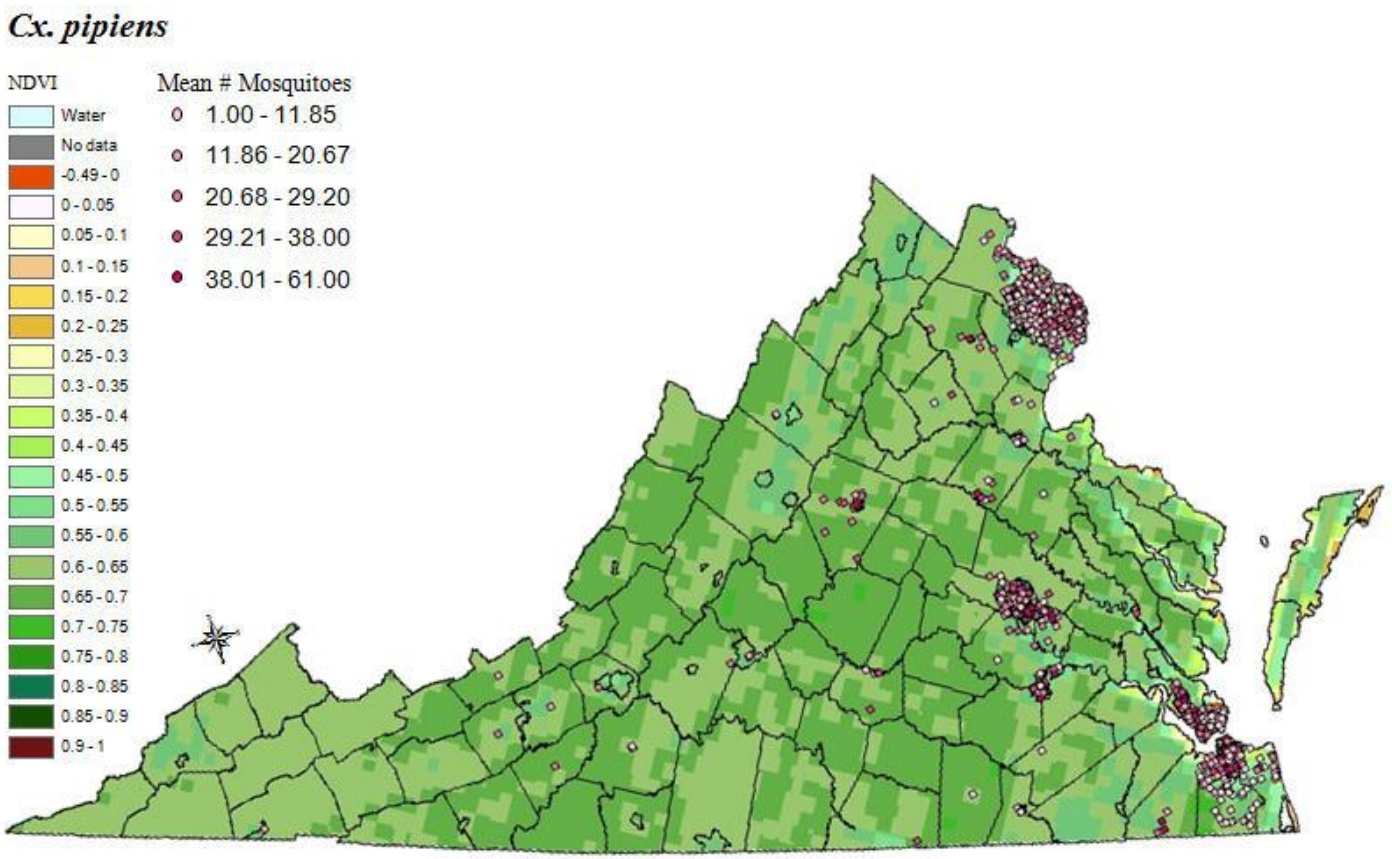

Figure A.7. *Cx. pipiens* distribution and abundance in Virginia.

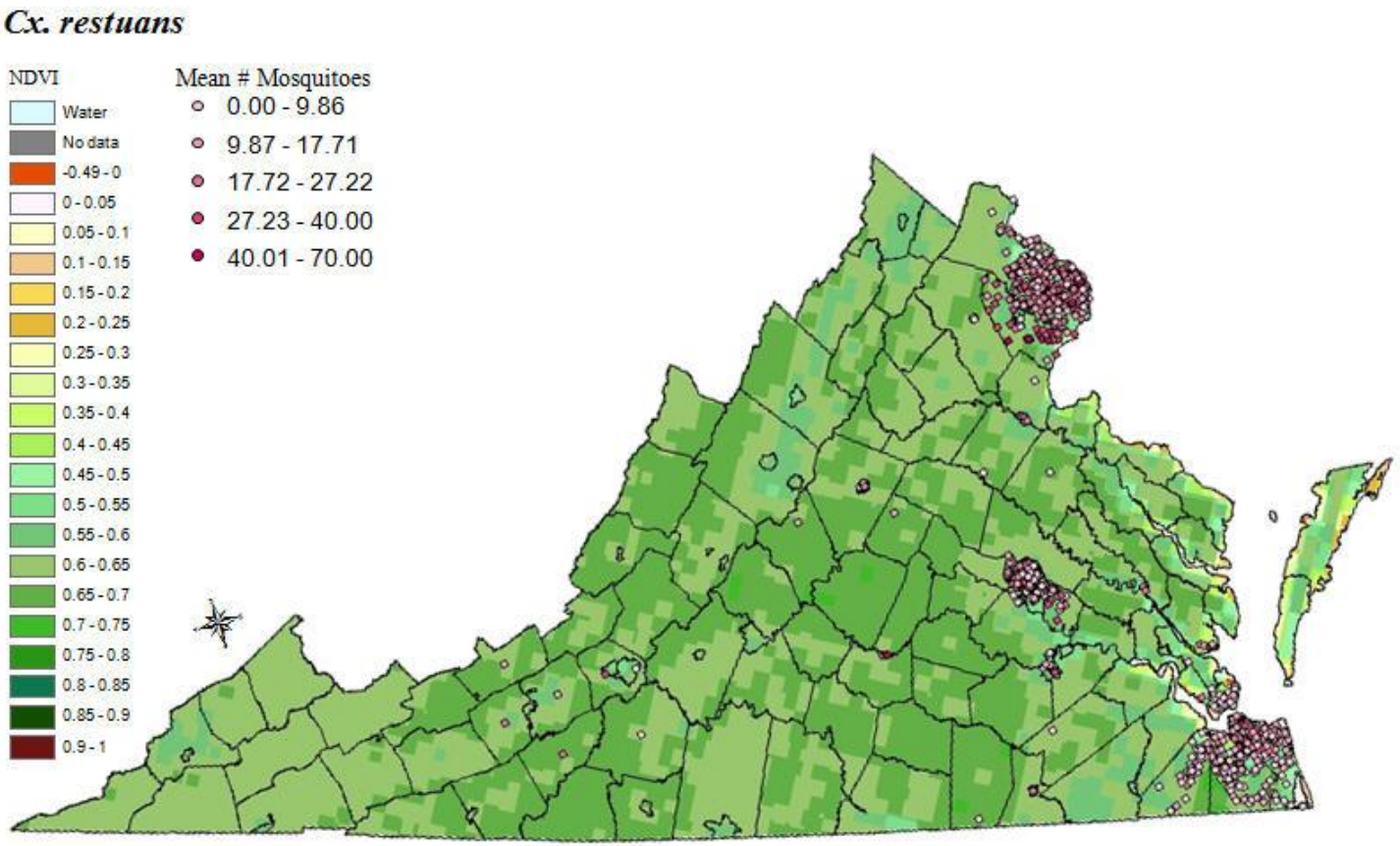

Figure A.8. *Cx. restuans* distribution and abundance in Virginia.

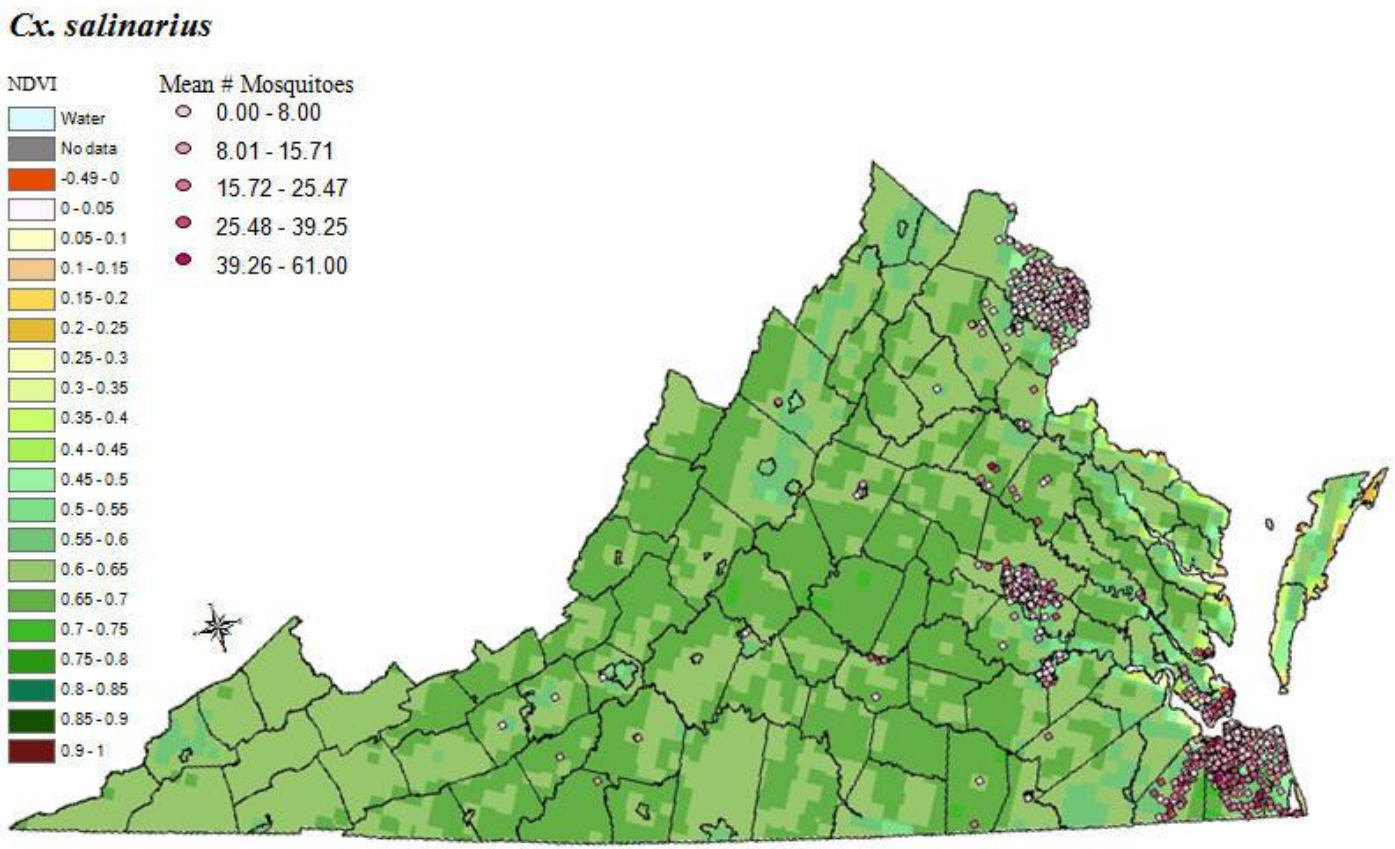

Figure A.9. *Cx. salinarius* distribution and abundance in Virginia.

APPENDIX B: GLOSSARY OF TERMS[1]

Agroterrorism – the deliberate introduction of a chemical or a disease agent, either against livestock/crops or into the food chain, for the purpose of undermining stability and/or generating fear (http://www.doacs.state.fl.us/aep/); intentional infection of livestock via infected animals, people, or vectors From CRS report: Agroterrorism is a subset of bioterrorism, and is defined as the deliberate introduction of an animal or plant disease with the goal of generating fear, causing economic loss, and/or undermining stability.

Antibiotic – a chemical derived from a fungus or bacteria which is inhibitory to other microorganisms.

Antigen – substance which induces an immune response upon contact with the immune system.

Arbovirus – a large heterogeneous group of RNA viruses divisible into groups on the basis of the virions; they have been recovered from arthropods, bats, and rodents; most are borne by arthropods; they are linked by the epidemiologic concept of transmission between vertebrate hosts by arthropod vectors (mosquitoes, ticks, sandflies, midges, etc.) that feed on blood; they can cause mild fevers, hepatitis, hemorrhagic fever, and encephalitis (www.dictionary.com, 30 Nov 2006).

Arthropod – member of the invertebrate phylum Arthropoda which includes insects, crustaceans, spiders, and ticks.

Biological transmission – involving a biological process, e.g. passing a stage of development of the infecting agent in an intermediate host. Opposite to mechanical transmission.

Biological vector – an arthropod vector in whose body the infecting organism develops or multiplies before becoming infective to the recipient individual.

Bioterrorism – the malicious use by terrorists of pathogens, parts of them, or their toxins in direct or indirect acts against humans, livestock or crops

Cholera – An acute infectious disease of the small intestine, caused by the bacterium *Vibrio cholerae* and characterized by profuse watery diarrhea, vomiting, muscle cramps, severe dehydration, and depletion of electrolytes.

Dengue fever – infectious disease caused by the dengue virus, which is transmitted by mosquitoes.

[1] The definitions presented here were obtained from www.dictionary.com except where otherwise noted.

Desiccation – drying.

Diapause – physiological state of suspended activity or arrested development that facilitates survival through a period of unfavorable conditions, but is initiated before the onset of these conditions (Bailey 1982).

Ecological niche – the conjunction of ecological conditions within which a species is able to maintain populations without immigration (Grinnell 1917; Holt and Gaines 1992).

Ecological niche model – an analytical tool based on a geographical information system that can incorporate remotely-sensed information about the environment offers the potential to define the limiting conditions for the disease in its native region for which there are some distributional data (Rogers and Randolph 2003).

Edema – swelling as a result of accumulation of fluid.

El Niño – a disruption of the ocean-atmosphere system in the Tropical Pacific having important consequences for weather and climate around the globe (EFSA 2005).

Emerging infectious disease – an infectious disease whose incidence has increased in the past 20 years and threatens to increase in the near future (Wikipedia Apr 2008).

Encephalitis – inflammation of the brain.

Endemic – a disease that is constantly present to a greater or lesser degree in a particular location.

Epidemic - a widespread outbreak of an infectious disease that affects many individuals in a population at the same time. Epidemics may be restricted to one locale, one region, or even the entire globe (pandemic). An epidemic is not a characterization of how many members or what proportion of the population is infected but is defined by how fast it is growing.

Epizootic – a disease which simultaneously attacks or is present in a large number of animals.

Establishment - the indigenous transmission of the disease by local vectors to sizable population.

Etiology – cause of disease.

Extrinsic incubation (EI) period – interval between host's ingestion of the virus and subsequent transmission through biting; varies for each virus and vector.

Febrile – relating to fever.

Geographic Information System – a computer system for capturing, storing, checking, integrating, manipulating, analysing and displaying data related to positions on the Earth's surface. Typically, a geographic information system (GIS) is used for handling maps of one kind or another. These might be represented as several different layers where each layer holds data about a particular kind of feature (e.g. roads). Each feature is linked to a position on the graphical image of a map. Layers of data are organised to be studied and to perform statistical analysis.

Georeference – to define existence in physical space; establish location in terms of map projections or coordinate systems.

Geospatial data – any electronic format data that contains both geometry (pixels and vectors) and the means to relate that geometry to a prescribed coordinate system. 'Page' units of measure are not acceptable in this definition. Often the data is referred to as GIS data. (From Virginia Department of Transportation)

Hemorrhagic – relating to or characterized by bleeding.

Hepatic – relating to the liver.

Host – organism from which infectious agent gains sustenance.

Indigenous – originating in a particular area.

Infectious diseases – caused by agents or pathogens, which are microorganisms. They can be spread to humans or animals, which are regarded as hosts, either directly or through a vector. The vector is usually an insect, such as ticks in Lyme disease or Rocky Mountain Spotted Fever, or mosquitoes in West Nile virus or Eastern Equine and other forms of encephalitis (Allen and Wong 2006).

Infectious feeding – introduction of the agent to the vector when the vector is feeding on the blood of an infected organism.

Infectious agent – the virus, bacteria, protozoan, or other microorganism which induces disease.

Infectivity threshold – concentration of virus that must be ingested by the vector in order for strains to become infective; varies among viruses and vectors.

Land-cover – the physical material (e.g., grass, asphalt, trees, bare ground, water) at the surface of the earth. There are two primary methods for capturing information on land cover: field survey and analysis of remotely-sensed imagery (Logicon 1997, Wikipedia 2009).

Larva – immature form of certain organisms (e.g., insects and ticks) which emerge from the egg.

Lyme disease – inflammatory infectious disease caused by the tick-transmitted spirochete, Borrelia burgdorferi.

Mechanical transmission - the transmitter is not infected in that tissues are not invaded and the agent does not multiply.

Mechanical vector - an arthropod vector that transmits the infective organisms from one host to another but is not essential to the life cycle of the parasite.

Meningeal – relating to the membranes surrounding the spinal cord and brain.

Meningitis – inflammation of the membranes of the brain and/or spinal cord.

Mesic – characterized by a moderate amount of moisture.

Nymph – immature form of certain organisms (e.g., ticks); the larva molts to become a nymph, which is adult like in form though usually smaller.

Pandemic – the outbreak of an infectious disease over a large geographical region and affecting a large percentage of the human and/or animal population.

Paresis – partial or incomplete paralysis.

Pathways assessment – systematic assessment of pathways along which a foreign animal disease might enter the United States and establish an outbreak of disease in animals and/or man – also applicable for delineating pathways for disease spread in the United States.

Plague – an infectious, epidemic disease caused by a bacterium, *Yersinia pestis*, characterized by fever, chills, and prostration, transmitted to humans from rats by means of the bites of fleas.

Remote sensing – the science of gathering data on an object or area from a considerable distance, as with radar or infrared photography, to observe the earth or a heavenly body.

Spatial autocorrelation - Statistical correlation between spatial random variables of the same type, attribute, name, etc., where the correlation depends on the distance and/or direction that separates the locations. Often we notice that locations that are nearby tend to have similar values – this is positive spatial autocorrelation.

Surveillance – activities involved in systematic collection, collation, and analysis of animal health data. **Disease surveillance** is an epidemiological practice by which the spread of disease is monitored in order to establish patterns of progression. The main role of disease surveillance is to predict, observe, and minimize the harm caused by outbreak, epidemic, and pandemic situations, as well as increase our knowledge as to what factors might contribute to such circumstances (Wikipedia 2008).

Systemic – relating to the organism as a whole.

Transovarial transmission – passage of infectious agent to eggs within the ovaries; larvae are subsequently infected (Mullen and Durden 2002).

Transstadial transmission – transmission of agent between different stages in the life history of an organism (Mullen and Durden 2002).

Vector – organism which transmits the infectious agent to the host; examples include ticks and mosquitoes (Mullen and Durden 2002).

Vector-borne disease – a disease in which the pathogenic microorganism is transmitted from an infected individual to another individual by an arthropod or other agent, sometimes with other animals serving as intermediary hosts (Mullen and Durden 2002).

Vectorial capacity - the overall ability of a vector species in a given location at a specific time to transmit a pathogen is known as vectorial capacity (Mullen and Durden 2002).

Viremia – the presence of a virus in the bloodstream.

Virulence – The relative degree or ability of a microorganism to cause disease or damage its host.

Virus – Any of various simple submicroscopic parasites of humans, animals, plants, and bacteria that are often pathogenic. Viruses consist essentially of a core piece of nucleic acid surrounded by a protein coat. Unable to replicate without a host cell, viruses are typically not considered living organisms.

West Nile virus – a member of the genus *Flavivirus*, family Flaviviridae, West Nile virus (WNV) is a vector-borne disease that has the potential to cause febrile illness, encephalitis, and occasionally death in humans (Theophilides *et al*. 2003; Brownstein *et al*. 2002, 2004; Glaser 2004; Hayes *et al*. 2005). The known cycle of transmission for WNV is from birds to mosquitoes to humans and horses, which are both considered dead-end hosts. However, the virus is believed to over-winter in mosquito populations and to reside within the bird 'reservoir' population (Allen and Wong 2006).

Vector competence – the vector competence of a species is a measure of how susceptible the vector is to infection with a pathogen and how efficiently that vector can transmit the pathogen to novel hosts. Vector competence can vary among populations of a given vector species and among species (Eldridge 2000).

Vector – borne infectious diseases are those diseases in which the infectious agent is transmitted to the human host via an agent -- the vector. The vectors for most of the diseases likely to be observed in the U.S. are arthropods, e.g., fleas, ticks, and mosquitoes. Notable examples of vector-borne diseases include malaria, which is transmitted to humans via mosquitoes, and bubonic plague, which is transmitted via infected fleas. (Plague is also transmitted directly from animals to animals, including humans, as a respiratory disease.)

Yellow fever – An infectious tropical disease caused by an arbovirus transmitted by mosquitoes of the genera *Aedes*, especially *A. aegypti*, and *Haemagogus* and characterized by high fever, jaundice, and often gastrointestinal hemorrhaging.

Zoonotic –A disease of animals that can be transmitted to humans.

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