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Evaluation Of The Current State Of Florida West Nile Surveillance Program As A Predictor For Control And Prevention Of Human West Nile Diseases

by

Angela E. Butler

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Public Health Department of Epidemiology College of Public Health University of South Florida

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November 19, 2004

Keywords: arboviruses, west nile, surveillance, vector, sentinel, avian

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# Table of Contents

List of Tables .................................................................................................................. iii
List of Figures .................................................................................................................. iv
List of Symbols and Abbreviations ................................................................................... x
Abstract ........................................................................................................................... xi

Introduction ....................................................................................................................... 1

Public Health Surveillance ................................................................................................. 1

Evaluation of the Arbosurveillance System in Florida .................................................... 4
  Description of Health Related Event ............................................................................ 4
  Distribution ..................................................................................................................... 4
  Transmission ................................................................................................................... 9

Clinical Presentation ......................................................................................................... 10

Florida’s Surveillance System ........................................................................................... 13

Florida’s Arbosurveillance Response Plan ........................................................................ 18

Defining the Stakeholders ............................................................................................... 21

Purpose and Objectives of System .................................................................................. 22

Evaluation Design ........................................................................................................... 22

Materials and Methods ..................................................................................................... 24

Analysis ............................................................................................................................. 26

Results ............................................................................................................................... 31

Demographic Analysis ....................................................................................................... 31
  Gender ............................................................................................................................. 31
  Age Groups ..................................................................................................................... 33
  West Nile Incidence Rates ............................................................................................ 36

Surveillance ......................................................................................................................... 39
  Temporal Distribution ..................................................................................................... 39

Clinical Cases ..................................................................................................................... 42

Sentinel Chicken Surveillance ........................................................................................... 44

Avian Surveillance ............................................................................................................... 46

Mosquito Surveillance ........................................................................................................ 53

Regional Surveillance ......................................................................................................... 58
  Panhandle Region .......................................................................................................... 58
  Northern Region .............................................................................................................. 62
  Central Region ................................................................................................................ 65
  Southern Region .............................................................................................................. 68
  Clinical Cases by Region ................................................................................................. 71

Spatial Analysis .................................................................................................................. 73
Positive Dead Birds and Clinical Cases .......................................................... 73
Positive Mosquito Pools and Clinical Cases .................................................. 84
Sentinel Chicken Serconversion Rates and Clinical Cases .......................... 95
Multivariate Poisson Regression Model .......................................................... 105

Discussion ........................................................................................................ 108

Demographic Analysis ..................................................................................... 108
Temporal Distribution ....................................................................................... 109
Peak Transmission ............................................................................................ 111
Regional Analysis .............................................................................................. 114
Spatial Analysis .................................................................................................. 118
Poisson Distribution Regression Model ............................................................ 119

Study Strengths and Limitations ..................................................................... 121
Evaluation ......................................................................................................... 122

References Cited ............................................................................................... 122

Bibliography ...................................................................................................... 129

Appendices ........................................................................................................ 132

Appendix I: Table of the Species of Birds that were found Positive for West Nile Virus in the United States since 1999 .................................................. 134
Appendix II: Table of the Species of Mosquitoes that were found Positive for West Nile Virus in Mosquito Pools in the United States since 1999 .......................................................... 142
Appendix III: Species Specific West Nile Virus Reservoir Competence Index Values .................................................................................................................. 143
Appendix VI: 2001 Data by County ................................................................ 144
Appendix V: 2002 Data by County ................................................................ 146
Appendix VI: 2003 Data by County ................................................................ 148
Appendix VII: Number of Sentinel Chicken Sites and Chickens per Site by Year... 150
Appendix VIII: County Arbo surveillance Table ................................................. 151
Appendix IX: Regional Map of Florida Numbered by Location from East (Right) to West (Left) and North (Top) to South (Bottom) .................................................. 153
Appendix X: Incidence Rate and Number of Clinical Cases per County by Year 154
Appendix XI: Graphs of Pooled Annual Averages (2001-2003) with Moving Averages for West Nile Surveillance Data Statewide and Regional per Week .................................................................................................................. 155
Appendix XII: PROC GENMOD Poisson Regression SAS Output ....................... 165
List of Tables

Table 1. Month of First Sera Submitted for Counties Participating in Sentinel Surveillance for 2001, 2002 and 2003 Organized by Region........................ 117
List of Figures

Figure 1. The Worldwide Geographic Distribution for the Serocomplex of the Family Flaviridae as of 2000. ................................................................. 6

Figure 2a. West Nile Virus Distribution across the United States from 1999 -2002... 7

Figure 2b. West Nile Virus Distribution across the United States for 2003. ............ 8

Figure 3. The Transmission Cycle for West Nile Virus. ....................................... 10

Figure 4. Diagram of the State of Florida’s Specific Response Plan for Arbovirus Detection. ........................................................................................................... 19

Figure 5. Total Number of West Nile Cases and Gender for 2001-2003................. 32

Figure 6. Cumulative Number of WN Cases for Gender by Year. .......................... 32

Figure 7. WNND by Age Group for 2001-2003. .................................................... 34

Figure 8. Age Comparison for WNND and WNF for 2003........................................ 34

Figure 9. WNND by Gender and Age Group for 2001-2003................................. 35

Figure 10a. West Nile Incidence Rate by County, Region and Location for 2001. ... 37

Figure 10b. West Nile Incidence Rate by County, Region and Location for 2002. ... 37

Figure 10c. West Nile Incidence Rate by County, Region and Location for 2003. ... 38

Figure 11. West Nile Clinical Statewide Incidence Rate for 2001 through 2003........ 38

Figure 12. Sentinel Chicken Positive West Nile Weekly Seroconversion Rate by Week for 2001 – 2003................................................................. 40

Figure 13. West Nile Clinical Cases by Week for 2001—2003............................ 40

Figure 14. Total Number of West Nile Positive Dead Birds by Week for 2001 - 2003 ................................................................. 41

Figure 15. Total Number of West Nile Positive Mosquito Pools by Week for 2001 - 2003 ................................................................. 41
Figure 16. Number of Clinical Cases of West Nile per Week during peak Transmission Season................................................................. 43
Figure 17. Box-plot for the Average and Total Number of Clinical Cases for 2001, 2002 and 2003................................................................. 43
Figure 18. Sentinel Chicken Surveillance Rate during Transmission Season for 2001 - 2003. ................................................................. 45
Figure 20a. Total Number of West Nile Positive Dead Birds during Transmission Season for 2001 – 2003................................................................. 49
Figure 20b. Dead Birds Percent Positive. ................................................................. 49
Figure : 21a. The Total Dead Birds Submitted and the Percent of Positive Dead Birds by Week for 2001................................................................. 50
Figure : 21b. The Total Dead Birds Submitted and the Percent of Positive Dead Birds by Week for 2002................................................................. 50
Figure : 21c. The Total Dead Birds Submitted and the Percent of Positive Dead Birds by Week for 2003................................................................. 51
Figure 22. Box-plot the Number of Positive Dead Birds for 2001, 2002 and 2003......................................................................................... 51
Figure 23. Total Dead Birds Submitted for 2001 – 2003.................................................. 52
Figure 24. Box-plot for Total Submitted Dead Birds for 2001, 2002 and 2003. ........ 52
Figure 25. Total Positive Mosquito Pools during Transmission Season for 2001 – 2003......................................................................................... 55
Figure 26. Percent of Positive Mosquito Pools during Transmission Season for 2001 – 2003......................................................................................... 55
Figure 27. Box-plot for Total Positive Mosquito Pools for 2001, 2002 and 2003......................................................................................... 56
Figure : 28a. The Total Positive Mosquito Pools and the Percent of Positive Mosquito Pools by Week for 2001. .................................................. 56
Figure : 28b. The Total Positive Mosquito Pools and the Percent of Positive Mosquito Pools by Week for 2002. .................................................. 57
Figure : 28c. The Total Positive Mosquito Pools and the Percent of Positive Mosquito Pools by Week for 2003. .......................................................... 57

Figure 29a. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Panhandle Region during 2001. ................................. 60

Figure 29b. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Panhandle Region during 2002. ................................. 60

Figure 29c. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Panhandle Region during 2003. ................................. 61

Figure 30a. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Northern Region during 2001. ................................. 63

Figure 30b. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Northern Region during 2002. ................................. 63

Figure 30c. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Northern Region during 2003. ................................. 64

Figure 31a. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Central Region during 2001. ................................. 66

Figure 31b. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Central Region during 2002. ................................. 66

Figure 31c. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Central Region during 2003. ................................. 67

Figure 32a. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Southern Region during 2001. ................................. 69

Figure 32b. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Southern Region during 2002. ................................. 69

Figure 32c. The Number of Positive Avian, Sentinels and Mosquitoes with Human Cases for the Southern Region during 2003. ................................. 70

Figure 33. Number of Clinical Cases by Region for 2001-2003 .......................... 72

Figure 34a. Florida West Nile Cumulative Positive Dead Bird Distribution and Clinical Cases by County for 2001-2003 ................................. 76

Figure 34b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for the Pooled Years 2001-2003. ............... 77
Figure 34c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for the Pooled Years 2001-2003 excluding outlier observations. ................................................................. 77

Figure 35a. Florida West Nile Positive Dead Bird Distribution and Clinical Cases by County for 2001. ................................................................. 78

Figure 35b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2001 ........................................ 79

Figure 36a. Florida West Nile Positive Dead Bird Distribution and Clinical Cases by County for 2002. ................................................................. 80

Figure 36b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2002 ........................................ 81

Figure 36c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2002 excluding outlier observations... 81

Figure 37a. Florida West Nile Positive Dead Avian Distribution and Clinical Cases by County for 2003. ................................................................. 82

Figure 37b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2003 ........................................ 83

Figure 37c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2003 ........................................ 83

Figure 38a. Florida West Nile Positive Mosquito Pool Distribution and Clinical Cases by County for 2001-2003. ................................................................. 87

Figure 38b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for the Pooled Years 2001-2003...... 88

Figure 38c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for the Pooled Years 2001-2003 excluding outlier observations. ......................................................... 88

Figure 39a. Florida West Nile Mosquito Pool Distribution and Clinical Cases by County for 2001. ................................................................. 88

Figure 39b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2001. ........................................ 90
Figure 39c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2001 excluding outlier observations. ................................................................. 90

Figure 40a. Florida West Nile Mosquito Pool Distribution and Clinical Cases by County for 2002. .................................................................................................................. 91

Figure 40b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2001. ................................................................. 92

Figure 40c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2001 excluding outlier observations. ................................................................. 92

Figure 41a. Florida West Nile Mosquito Pool Distribution and Clinical Cases by County for 2003. .................................................................................................................. 93

Figure 41b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2003. ................................................................. 94

Figure 41c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2003 excluding outlier observations. ................................................................. 94

Figure 42a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2001-2003......................... 97

Figure 42b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for the Pooled Years 2001-2003. ................................................................. 98

Figure 42c. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for the Pooled Years 2001-2003 excluding outlier observations................................................................. 98

Figure 43a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2001......................... 99

Figure 43b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2001................................. 100

Figure 44a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2002......................... 101

Figure 44b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2002................................. 102
Figure 45a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2003. .......................... 103

Figure 45b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2003. ............................... 104

Figure 45c. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2003 excluding outlier observations. ........................................................................ 104

Figure 46: Epi Curve Comparing 2003 and 2004 Confirmed Human Cases. ........ 111
## List of Symbols and Abbreviations

<table>
<thead>
<tr>
<th>Symbol and Abbreviations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>BoEPC</td>
<td>Bureau of Entomology and Pest Control</td>
</tr>
<tr>
<td>BOL</td>
<td>Bureau of Laboratories</td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act of 1996</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CHD</td>
<td>County Health Department</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>DACS</td>
<td>Department of Agriculture and Consumer Services</td>
</tr>
<tr>
<td>DAI</td>
<td>Division of Animal Industry</td>
</tr>
<tr>
<td>DEP</td>
<td>Department of Environmental Protection</td>
</tr>
<tr>
<td>FBE</td>
<td>Florida Bureau of Epidemiology</td>
</tr>
<tr>
<td>FDOH</td>
<td>Florida Department of Health</td>
</tr>
<tr>
<td>FWCC</td>
<td>Florida Wildlife Conservation Commission</td>
</tr>
<tr>
<td>HAI</td>
<td>Hemagglutination Inhibition Assay</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>MAC-ELISA</td>
<td>IgM Antibody Capture Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>PRNT</td>
<td>Serum Neutralization Plaque Reduction Test</td>
</tr>
<tr>
<td>WN</td>
<td>West Nile</td>
</tr>
<tr>
<td>WNF</td>
<td>West Nile Fever</td>
</tr>
<tr>
<td>WNND</td>
<td>West Nile Neuroinvasive Disease</td>
</tr>
<tr>
<td>WNV</td>
<td>West Nile virus</td>
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</table>
West Nile is an important novel virus in the United States having spread rapidly since it was first detected in New York in 1999. The Centers for Disease Control and Prevention as well as, many State Health Departments have mandated programs for surveillance of West Nile Virus activity. These programs incorporate many different aspects including existing arboserology programs with additional testing for West Nile Virus and new plans that incorporate active and passive surveillance methods.

The objective of this study was to examine all aspects of the Florida West Nile surveillance program to determine if there was transmission in the animal systems prior to human cases. The predictive analyses were done using regional data graphs, spatial information, correlations and regression models.

Data for sentinel chickens, bird necropsy and mosquito pool surveillance from participating counties in Florida were obtained from the State of Florida surveillance database. The human data was obtained from the State of Florida reportable disease database for each county whether participating in the state surveillance programs or not. Clinical cases were examined by demographics (gender and age) and an incidence rate
was calculated to demonstrate the effects of disease. Specific statistical methods used included Pearson’s coefficient correlation, Poisson distribution regression modeling to show if any of the surveillance systems were predictors for human disease.

The incidence rate analysis for clinical cases showed clustering of cases in adjacent counties with in a region where Florida’s panhandle and adjacent counties northeast had the highest incidence. Florida’s central and southern regions had moderate human incidence. This provides useful information in transmission geography for prevention and control measures. Demographic analysis showed that there were twice as many males then females diagnosed with West Nile in Florida, this was true across the groups as well. The highest number of cases was seen within the age group over 55 years of age for West Nile Neuroinvasive Disease and for West Nile Fever the highest number of cases was within the 36-54 age range.

The temporal distribution was determined using graphical representations of the all of the surveillance types and clinical cases. In order to include all relevant data the temporality was set from week 20 to week 52. This study found that all of the surveillance types (dead birds, mosquitoes and sentinels) offered a specialized strength for predicting clinical cases. However, mosquitoes proved to be the least efficient out of the three surveillance systems. The regional and spatial analysis showed that positive dead birds and sentinels provided the coverage for the surveillance systems in the state. However, Pearson’s correlation coefficient was low for sentinel surveillance; this may be due to higher participation showing West Nile Virus activity in areas (especially rural) that have no reported human cases. This analysis did show that West Nile is detected in mosquito pool samples before it is detected in the dead bird or sentinel surveillance
systems which provides an earlier warning for human cases. The Poisson distribution regression model was only useful for the pooled years and 2003. These showed that mosquitoes, positive dead birds and sentinels were good predictors for clinical cases for the combined years and dead birds and sentinels were significant for 2003 as well. The recommendations based on the results from this study would be to continue all the current surveillance efforts but with the following enhancements: 1. Increase the coverage and consistency of submissions for all surveillance types. 2. Set standard levels of participation for all counties based on the regional analyses and populations at risk. 3. Create standardized approaches for sampling, shipping and submitting samples (especially for mosquito pool submissions) and require that participating counties adhere to these standards. 4. Only submit specific birds known to be especially susceptible to West Nile Virus (e.g. corvids). 5. Targeted prevention and education strategies for higher risk groups based on their potential levels of exposure.
Introduction

Public Health Surveillance

Public health surveillance is defined as the continuous systematic collection, analysis, interpretation and dissemination of data regarding a health-related event, which is used for public health actions to reduce morbidity, mortality and improve the overall health of the population (CDC, 2001b). An effective surveillance program should incorporate five essential elements: 1) a precisely defined population and disease case definition, 2) standardized data collection, consolidation and evaluation methods, 3) proper analysis tools to correctly interpret information, 4) a feedback system to disseminate information so that the target population is aware of public health concerns and 5) timely response and implementation of changes to public health practices that reflect the information gained during surveillance activities. The application of surveillance activities support case detection and public health interventions, provides an estimate of the impact of disease, defines the natural history of a health condition, determines the distribution and spread of illness, generates hypotheses and stimulates research, evaluates prevention and control measures and helps facilitate program planning (Teutsh, 2000).

Perhaps the most important function of public health surveillance is outbreak detection – identifying an increase in the frequency of a health-related event above the
background occurrence of that event both timely and accurately (Broom, 2004). Early
detection of outbreaks can be achieved by assuring timely and complete receipt, review,
and follow-up of disease case reports, responding to slight indications that possible
events of interest are occurring (i.e., lowering the threshold for investigating possible
outbreaks, or using modeling tools to improve the predictive value of current programs to
identify an outbreak at an earlier stage), and by monitoring new types of data that may
indicate an outbreak event earlier than current surveillance data (Broom, 2004). In order
to adequately identify or predict outbreaks, a baseline (background) threshold should be
set to better recognize epidemics. An epidemic is relative to the frequency of the disease
within a defined population during a particular season of the year. Epidemics are usually
characterized by an increase in cases showing two standard deviations above the mean.
Consequently, one case of a disease normally absent or not previously recognized in a
specified area may not be sufficient to signify an epidemic; whereas two cases in the
same area may be adequate (Last, 2001). By setting a threshold limit, levels of higher
than normal activity can therefore be determined. This can help to provide a more
accurate basis for implementing preventive and control measures.

The most critical challenge to public health surveillance is maintaining the
efficacy of the program. In 1988, the Centers for Disease Control and Prevention (CDC)
published *Guidelines for Evaluating Surveillance Systems* to promote the best use of
public health resources through the development of efficient and effective public health
surveillance systems. These guidelines were updated in 2001 to address the need for a)
the integration of surveillance and health information systems, b) the establishment of
data standards, c) the electronic exchange of health data, and d) changes in the objectives
of public health surveillance to facilitate the response of public health to emerging health threats. As a supplement to these publications, the CDC published *Framework for Evaluating Public Health Surveillance Systems for Early Detection of Outbreaks* in 2004 for the purposes of evaluating public health surveillance systems for their timely detection of outbreaks. The purpose of evaluating public health surveillance systems is to ensure that problems of public health importance are being monitored efficiently and effectively. Public health surveillance systems should be evaluated periodically, to improve the quality, efficiency, and usefulness of the program and should focus on how well the system operates to meet its purpose and objectives. A public health surveillance system should emphasize the components that are most important for the objectives of the system. An evaluation of that system must therefore consider the same components (CDC, 2001c). In order to establish the relative value of different approaches and improve early detection of outbreak efficacy, there must be a focused attention to the measurement of the performance of public health surveillance systems (Broom, 2004).

The *Updated Guidelines for Evaluating Surveillance Systems and Framework for Evaluating Public Health Surveillance Systems for Early Detection of Outbreaks* describe four main tasks involved in evaluating public health surveillance systems for early outbreak detection. The first is a description of the surveillance system being evaluated including: defining the stakeholders (individuals or agencies who provide and use the information generated by the system), the public health importance of the health-related event under surveillance (frequency, severity, preventability and public interest), a description of the purpose and operation of the surveillance system (purpose and objectives of the system), planned uses of the data collected, health-related event under
surveillance with case definition, a response flow chart, and a description of the resources used to operate the surveillance system. The second task focuses on the evaluation design by determining the specific purpose of the evaluation, considering what will be done with the information generated from the evaluation, and specifying the questions that will be answered by the evaluation. The third task evaluates the performance of the surveillance system by indicating the level of usefulness and describing the system’s simplicity, flexibility, acceptability, and stability. The final task draws conclusions from the evaluation and recommends new uses and improvements to the system (CDC, 2001c; Broom, 2004).

**Evaluation of the Arbosurveillance System in Florida**

*Description of Health Related Event*

West Nile Virus (WNV) is a single-stranded RNA virus in the family *Flaviridae*, genus *Flavivirus*. It is an arbovirus (Arthropod-Borne-Virus) primarily transmitted by mosquitoes. The virus can infect a wide range of hosts including humans, birds and horses. WNV has also been isolated in other mammals and alligators, however, little is known about the associated symptomology for these infections (Stark, 2003).

*Distribution*

West Nile Virus has been characterized with worldwide outbreaks every few years with the temporal distribution primarily during late summer and fall. It was first identified in the West Nile district of Uganda in 1937 (Huhn, 2003), but it wasn’t until 1957, during an outbreak in Israel, that it was recognized as a cause of severe human meningoencephalitis (Chowers, 2000). Descriptions of disease symptoms in Israel can be
found dating back to the early 1940’s. This connection linking WNV and Israel may be
due to the fact that many bird species have migratory patterns with their fly way zone
from Europe to Africa through Israel (Leffkowitz, 1942). In the 1960’s WNV was
associated with equine illnesses in France and Egypt (Murgue, 2000; Halouzka, 1999)
and began to threaten the United States in 1999 (Komar, 1999; Nasci, 1999; Nash, 2001).

Worldwide distribution is limited by ecological patterns supporting the WNV
transmission cycle. These limiting factors include temperature, precipitation levels and
vegetation all of which influence the vector and host relationship (Gubler, 2001). Figure
1 shows the worldwide distribution for flaviviruses as of 2000 where WNV was primarily
found in parts of Africa, Europe and parts of Asia with the exception of New York in the
United States.
Figure 1. The Worldwide Geographic Distribution for the Serocomplex of the Family Flaviridae as of 2000. This map shows West Nile Virus primarily in Africa and Europe however the New York outbreak is indicated.

West Nile Virus emerged as a public health threat in the United States in 1999 with an outbreak in New York (CDC, 1999; Komar, 1999; Nasci, 1999; Nash, 1999), and has since spread throughout North America. Figure 2a shows the distribution of West
Nile Virus activity as of 2002 and Figure 2b shows additional activity from 2003.

Confirmed cases of WN disease in humans has been reported in 45 states and the District of Columbia (CDC, 2003b) with evidence of enzootic activity (natural transmission cycle between mosquitoes and avian hosts) in 28 states (Huhn, 2003).

**Figure 2a. West Nile Virus Distribution across the United States from 1999-2002.**
This map shows the spread by year from the first appearance of WN in New York through 2002. The states colored white have had no WN activity as of December 2002.

*Map source: CDC website. Available from URL http://www.cdc.gov/ncidod/dvbid/westnile/surv&control03Maps99_02.htm*
Figure 2b. West Nile Virus Distribution across the United States for 2003.
This map shows the distribution of human WN disease as well as bird, animal and mosquito infections. The states colored white have not had any WN activity as of December 2003.


In Florida, WNV was first isolated in July of 2001 in a crow from Jefferson County located in the panhandle region (Blackmore, 2001). As of December, 2003 there have been a total 140 laboratory confirmed cases of WN disease in the human population across 44 of Florida’s 67 counties (Stark, 2003).
Transmission

Certain wild avian populations are considered the primary hosts for WNV maintaining a natural enzootic cycle with mosquitoes. The passerines (song birds) have been found to be the most competent primary hosts by supporting a viremia high enough to infect feeding mosquitoes; however some of these species do not have long enough sustained life. The most competent primary host which supports both a high enough viremia and a long enough sustained life is most likely the House Finch (Komar, 2003). The specific species, their associated viremia and sustained life span are shown in Appendix III. Like other viruses in its family, WNV infections in humans and other animals (including some bird species) are considered to be dead-end or incidental hosts because viremia is not sufficient to support transmission (Hadler, 2000; Huhn, 2003). The primary enzootic cycle is maintained by the presence of the vector and the host and epizootic cycle requires interaction with the bridge vector mosquito and an “incidental” human host. The mosquitoes that are part of the cycle tend to be ornithophilic (e.g. *Culex pipiens*) (Tyler, 2001; CDC, 2002). There have been several documented cases where person-to-person transmission has occurred, however, through organ transplants, blood transfusion and breast-feeding (Mitka, 2003). Human cases of WNV infection are generally low in number until daily rainfall patterns stimulate mosquito vectors to become more active (FDEH, 2003). It should be noted however, that a hallmark of outbreaks located in the United States had significant mortality within population of corvid species (crow and blue jay) serving as amplifying hosts (Eidson et al., 2000a; Bernard, 2000; Eidson et al., 2000b). However, corvids tend to die rapidly after they are
infected with WNV leading to a debate on whether they can truly be the primary
amplifying host. A detailed picture of the transmission cycles is depicted in Figure 3.

**Figure 3. The Transmission Cycle for West Nile Virus.**
The diagram shows the vector/bird primary enzootic cycle and transmission to “incidental” hosts by a bridge vector mosquito. The incidental hosts include horses, humans and other animals.

*Diagram used with permission from David Klemm.

**Clinical Presentation**

Following transmission of the virus by the mosquito vector, WNV multiplies in
the host’s circulatory system and may cross the blood-brain barrier to reach the central
nervous system (CNS). The virus generally requires an incubation period of 3-14 days
and symptoms can last from 3 to 6 days (CDC, 2001a; Campbell, 2002). Infection of the
brain interferes with normal CNS function and begins to cause inflammation of the
tissues surrounding the brain (Sejvar, 2003). The severity of the infection depends on the
host’s immune response to viral replication. People over 50 years of age are more likely
to develop sever symptoms associated with West Nile infection, but severe disease can
occur in individuals of any age (Campbell, 2002; Petersen, 2002). It is unknown whether
immunocompromised persons are at an increased risk for WNV associated infections (Peterson, 2003).

Most WNV infections are sub-clinical, with only about 20% of infected individuals developing a mild form of disease termed WNF. Due to under-reporting, a complete clinical picture of WNF’s effects on the population of the United States has not yet been determined, but it is characterized as a febrile illness of sudden onset with generalized flu-like symptoms including malaise, anorexia, nausea, vomiting, eye pain, headache, myalgia, rash and lymphadenopathy (CDC, 2001a).

A more severe neurological disease may develop in approximately 1 out 150 people infected with West Nile. West Nile Neuro-invasive Disease (WNND) is classified as a meningoencephalitis (inflammation of the brain and surrounding membranes) and occurs most often in patients of advanced age (> 50 years old). Recent outbreaks have described a hallmark fever, severe muscle weakness ranging to flaccid paralysis (rare), ataxia, gastrointestinal upset, changes in mental status and death. More severe neurological presentations have included seizures, myelitis, cranial nerve deterioration, optic neuritis, and polyradiculitis. Rarely, a maculopapular or morbilliform rash forming on the neck, trunk, arms or legs will develop with severe disease (CDC, 2001a).

Treatment of WNV associated illnesses, whether mild or severe, is typically supportive. Often, the patient will be hospitalized and given intravenous fluids, respiratory support and antibiotics for the prevention of secondary infections (CDC, 2001a).

The Centers for Disease Control and Prevention’s case definition for WNND includes probable and confirmed cases. A probable case is determined by the presence of
encephalitis or meningitis during arboviral transmission season and serology showing an elevated titer of virus-specific serum antibodies (less than a two-fold increase) or serum IgM or IgG antibodies detected by antibody-capture EIA with no available results for the same or later specimen of virus-specific serum antibodies. A probable case will be considered a confirmed case when tests serologically confirm a four fold increase in virus-specific antibodies between acute and convalescent sera usually collected two weeks apart (CDC, 2003a; Marfin, 2001) Based on this case definition a confirmed case of WNND may be diagnosed in Florida after one of following laboratory criteria has been met: a fourfold or greater change in WNV-specific serum antibody titer, or the isolation of WNV from or demonstration of West Nile viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, or the presence of specific IgM antibody by enzyme immunoassay (EIA) antibody capture in CSF or serum with confirmation by IgG-EIA or another serologic assay (e.g. neutralization or hemagglutination inhibition (HAI)). Both acute and convalescent sera from reported and suspected cases should be drawn since cross-reactivity between WNV and other closely related flaviviruses can occur if the patient has recently been vaccinated against a flavivirus (e.g., Yellow Fever) or infected with another flavivirus and may present a false positive West Nile MAC-ELISA result (FDEH, 2003).

West Nile Neuro-invasive Disease is a mandatory state reportable disease under Section 381.0031.of the Florida Statute. Therefore, all probable and confirmed cases of WNND must be reported to the Florida Department of Health (FDOH).

Diagnosis of WNND usually begins when a patient presents with an encephalitis or meningitis of unknown origin. Upon receipt of this evidence an immediate
epidemiological investigation for arboviral infection should be conducted. The Human Case Investigation Guidelines set forth in *Surveillance and Control of Selected Arthropod-borne Diseases in Florida 2003* recommends conducting a case interview which includes the age of the patient, history of mosquito bites within 14 days prior to the onset of symptoms, travel and activity history that would increase the risk of an arboviral illness, an environmental investigation to determine the risk of mosquito activity and local enzootic transmission or other regional human cases. Neurological symptoms caused by arboviruses mimic symptoms of most other CNS infections. Therefore, appropriate specimens should be collected and sent to the Florida Department of Health (FDOH) Bureau of Laboratories (BOL) for a confirmed diagnosis.

**Florida’s Surveillance System**

A comprehensive surveillance program for arboviruses should consist of monitoring for increases in arboviral seroconversion rates in sentinel chickens, weather patterns, the presence of vector and amplification host species (based on species infection rates), and the incidence of human and animal disease (FDEH, 2003).

Florida’s existing arbosurveillance plan involves several distinct monitoring systems including the vectors, a wide variety of hosts and captive sentinels. The hosts include serosurveillance of sentinel chicken flocks, wild-avian sero-survey and detection of the virus through molecular and isolation methods for the vectors and laboratory submitted dead avian specimen. Veterinary surveillance is passive and detection is also indicated by molecular methods and isolation from the tissue of submitted animals. Serological testing for veterinary surveillance is also done at the Kissimmee Department of Agriculture and Consumer Services (DACS) lab.
The entire surveillance plan encompasses several laboratory methods for determining the presence of WNV: Hemagglutination Inhibition Assays (HAI), IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (ELISA), Serum Neutralization-Plaque Reduction Assay (SNPR), Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and virus isolation in cell culture. These collectively are the most common detection methods used in WN surveillance.

The HIA is used to detect antibodies for a specified virus (i.e. flaviviruses) in sera from a wide variety of animals (most commonly humans, chickens and horses). HAI is used as an initial screening assay for sentinel surveillance because it can efficiently test a large number of samples from diverse species. Confirmations for sentinel chicken positives or reactive samples are assayed using the IgM Antibody Capture ELISA which is more specific than the HAI test. Confirmations for non-chicken samples are confirmed with SNPR.

Animal tissues are tested for the presence of West Nile viral RNA using the RT-PCR and virus culture for isolation. This is utilized for WNV detection in avian surveillance and mosquito surveillance to detect virus activity from submitting counties.

Viral amplification and transmission in the environment can be deduced using the arboviral surveillance system for detection, which may also indicate risks of human disease.

Due to the complex nature of the arboviral transmission cycles, multiple surveillance and detection methods are necessary for an accurate risk assessment. This is achieved by providing specific threshold levels, indicator parameters and predictive models, all of which may vary by season and region. The surveillance system in Florida
consists chiefly of avian mortality and morbidity, sentinel chicken flocks, and vector
testing. These samples are submitted to the Florida Department of Health, Tampa Branch
Bureau of Laboratories, but program compliance is reliant on individual county Mosquito
Control Districts and County Health Departments. Equine surveillance is also included as
a component of West Nile surveillance. Testing is generally done by DACS however; it
is only performed for suspected cases. Some Mosquito Control districts do their own
mosquito pool testing using traditional Polymerase Chain Reaction (PCR), Vectests and
Ramp tests. The data from these tests are not reported to the Department of Health;
therefore, outcomes are not included for prevention and control measures mandated by
the state.

Bird mortality surveillance is used to detect West Nile activity within a particular
geographical area. Through reporting and testing of dead birds, mortality patterns among
avian species (particularly corvids) may be utilized to generate a spatial analysis to help
determine a specific geographical area at risk for human cases of WNV associated
disease (Eidson, 2000a). This is only useful, however, when looking at mortality among
non-migratory bird species. Laboratory testing of dead bird tissues is necessary for an
accurate picture of viral presence in a specific geographical area. Collection, shipping and
laboratory activities are time consuming and costly; however, posing a major challenge to
this type of surveillance. A recent study on the economic impact of WN disease in
Louisiana during 2002 showed an overall cost of 20.1 million dollars from overall
medical expenses for 342 cases (Armineh, 2004). Another area of concern when using
this data to predict the risk of human disease is the mobility of individual birds. For
example, geographic analyses can be skewed when a dead bird is submitted from an area
other than where it was initially infected. Spatial analysis from reports of dead birds tends to work better in urban areas of Florida (e.g., North Florida) where dead birds are correlated with human population density, as opposed to rural areas.

Serosurveillance of captive sentinel flocks reduces these concerns. Ideally, a sentinel flock would be susceptible to infection but resistant to the disease thus minimizing their contribution to the transmission cycle. Sentinels should develop a rapid immune response and seroconvert before the disease can be detected within the human population. Lastly, they must be easily maintained with minimal health risks to their handlers (Komar, 2001). Florida uses chickens as their sentinel species since they maintain most of the attributes of an ideal sentinel. They have provided a good model for local activity of arboviruses. Chickens are placed in cages on sites located throughout the state to allow natural transmission between the vector and the host. The sites are chosen based either on the geographical availability or because of past activity in an area. The overall goal is to indicate WN activity within a geographical area through seroconversion rates of the sentinel chickens.

Several mammal models have been used, ranging from equines and canines to several different species of rodents (Komar, 2001). Theoretically, sentinel mammals would be a better representation of the epizootic transmission cycle rather than the enzootic cycle among birds. Therefore, captive mammals used as sentinels may be a better gauge for the risk of human disease. In Florida horses were initially looked at as a sentinel mammal but it proved too costly to maintain these herds.

Mosquito Control Districts and some private special taxing districts are the controlling entities of the county mosquito control in which they are located, and
participation in surveillance activities is voluntary. While some counties participate regularly in surveillance activities most do not. Florida has not had much success with mosquito surveillance however; some states (e.g., Colorado) with higher viral activity and submittal rates have had more success with this type of surveillance. If areas in Florida were to have an increase in transmission or more submissions during peak periods of activity for the affected geographical areas, mosquitoes may show better predictive value for forecasting trends in clinical West Nile cases.

Several collection techniques are compatible with laboratory testing; those available to the mosquito control districts include CDC traps (with or without Co²), Gravid traps, ABC light traps (with Co²), MM-X traps (with CO²), Lardcan and Mosquito Magnet traps. These traps may be placed anywhere WNV transmission is suspected. Additional trap sites can be added as needed, and should be considered in areas of past viral activity or where avian reservoirs or sentinel flocks are located. Male mosquitoes do not take blood meals and are incapable of transmitting WNV, therefore, mosquito pools sent to the laboratory for testing should consist of non-fed (because it would be impossible to differentiate whether the virus originated with the mosquito or the blood meal), gravid, female mosquitoes only (FDEH, 2004).

Collection of mosquito pools by the county mosquito control districts are logged by collection site, date and species of mosquito then shipped to the laboratory for testing with RT-PCR and viral isolation for the presence of WNV.

Florida also uses passive veterinary surveillance for the detection of West Nile in other animals. This type of surveillance requires that veterinarians send tissue, blood or CSF from suspected animal cases of West Nile to the laboratory for testing. The tissues
are assayed through molecular testing and isolation by the same protocols as avian and mosquito samples. Unfortunately, data collected from passive surveillance efforts is traditionally unreliable due to poor compliance thus, does not provide useful information for use as a predictive model in human cases.

**Florida’s Arbosurveillance Response Plan**

The state of Florida has set up a response plan for Mosquito-Borne (arboviruses) Diseases (figure 4) based on the continuous results from the arbosurveillance efforts. The plan has four tiers: Background Activity, Mosquito-Borne Illness Advisory, Mosquito-Borne Illness Alert, and Mosquito-Borne Illness Threat. Each tier includes specific criteria, geographical areas and response efforts. Since transmission generally occurs locally, the response plan is proposed for the affected counties or regions and is not intended as a statewide response plan (FDEH, 2004).
Figure 4. Diagram of the State of Florida’s Specific Response Plan for Arbovirus Detection.
This includes different advisories for a specific percentage increase among the surveillance systems.
Background activity occurs when the percentage of positives within the surveillance system does not exceed historical levels for that region. The response for background activity would be continued regular surveillance efforts (FDEH, 2004).

A Mosquito-Borne Illness Advisory, the second response tier, will be declared when the surveillance in a particular geographic area reveals a 10% increase in sentinel seroconversions, a 10% increase in corvid mortality, a 10% increase in the minimal infection rate (MIR) of vector mosquitoes all above normal background activity or two or more confirmed equine cases during two consecutive weeks. This would indicate a potential increase in viral activity thus increasing the risk of human infections. The overall response should include continued surveillance activities with the addition of public information announcements and health care provider advisories (FEDH, 2004).

The third tier, Mosquito-Borne Illness Alert, is initiated with a confirmed clinical case or 50% increase in sentinel seroconversions within a county or flock, or a 50% increase in corvid mortality. A mosquito-borne illness alert response should include the continuation of the previous responses for background activity and Mosquito-Borne Illness Advisory while increasing mosquito control measures on the local level.

The last tier, Mosquito-Borne Illness Threat, will be invoked if there is a potential for widespread clinical disease associated with arboviral infection and will be declared by the State Health Officer (FDEH, 2004).

In addition to Florida’s Response Plan for Mosquito-Borne Diseases, the state maintains an ongoing program for the prevention of transmission to humans. The existing arboviral prevention campaign in Florida includes education on the “5 D’s of Prevention:
“Dusk and Dawn, Dress, DEET, and Drainage” and community intervention through medical alerts issued when surveillance systems indicate increases arboviral activity.

Currently, no vaccine is available for the prevention of WNV, but clinical trials are in progress (WHO, 2004). The CDC also has a national program called “Fight the Bite” which is based on the same strategies as Florida’s prevention campaign (CDC, 2004).

**Defining the Stakeholders**

The State of Florida defines its primary stakeholders as “interagency partners” taken from both the state and local levels. These partners are responsible for coordinating the dissemination of information to the proper parties. DACS Bureau of Entomology and Pest Control (BoEPC) is responsible for notifying all mosquito control agencies for the affected counties and DACS Division of Animal Industry (DAI) will notify animal industry organizations and veterinarians. The Florida Wildlife Conservation Commission (FWCC) and the Department of Environmental Protection (DEP) will notify regional biologists and wildlife rehabilitators. The FDOH, BOL will notify the County Health Departments (CHD), the Department of Community and Environmental Health, CDC and sample submitters. The FDOH and CHDs are also responsible for the release of all public information regarding health alerts to physicians and hospitals and recommended precautions, while the local mosquito control agencies (or BoEPC if none exist) are responsible for the release of information regarding mosquito control activities (FDEH, 2003).
**Purpose and Objectives of System**

*The Surveillance and Control of Selected Arthropod-borne Diseases in Florida*

2003 published by the Florida Department of Health, Division of Environmental Health establishes guidelines for detecting and monitoring arthropod-borne diseases such as West Nile Neuro-invasive Disease (WNND) and WNF to minimize the risk of human infection. Its purpose is two-fold. First, to identify the system functions, such as surveillance and data management activities, that monitor sentinel chicken flocks, wild bird and mosquito populations. The second is to propose prompt and effective control methods. Such a program would eventually allow the State of Florida to determine the likelihood of the location and time of possible arboviral transmission to humans prior to an outbreak event, allowing time to implement the necessary preventive procedures.

The data collected from these surveillance activities should be used to further characterize the threat of arthropod-borne diseases in Florida and the United States, provide adequate prevention and control of arthropod-borne diseases during peak transmission periods, and improve the overall health and well being of the general population in these areas.

**Evaluation Design**

The unique environmental and demographic conditions found in Florida create an increased risk of exposure and a potential increased risk of contracting the diseases associated with West Nile Infection. The State of Florida has an extensive ongoing surveillance program for the detection of arboviruses. The overall outcome of each disease monitoring activity included in the surveillance program should either
independently or collectively act as a warning system to prevent human WNV Disease via various medical alerts, increased mosquito control and heightened public awareness through educational efforts. Early detection of outbreak conditions through surveillance could become an important public health control and prevention strategy for human illness associated with West Nile virus.

This study evaluated the current arbosurveillance programs for the detection of WNND and WNF cases in the state of Florida. While evaluating the data collected from these surveillance programs, this study attempted to develop a model for the early detection of human West Nile infections. Questions that this evaluation answers include: will the data collected by Florida’s Arthropod-borne Disease Surveillance Program provide the appropriate information necessary for the generation of a model to predict human West Nile virus infection, which disease monitoring effort or combination of efforts included within the evaluation yielded the most significant data for use with the model, and will this model provide an accurate and early warning of human disease associated with West Nile infection?

The information generated from this evaluation was used strictly to determine whether a model for predicting human disease is possible and whether that model could serve as an accurate predictor of human cases.
Materials and Methods

Four datasets were compiled for this evaluation: human, sentinel chicken, dead birds and mosquito and are summarized for each year (2001, 2002 and 2003) in Appendices IV, V and VI respectively. The Florida Department of Health (FDOH), Division of Environmental Health, provided the information contained in the human dataset. Sentinel chicken, dead birds and mosquito data were all obtained through the FDOH, Bureau of Laboratories.

The human dataset consists of all confirmed WNND and WNF cases reported to the FDOH from 2001 to 2003. The year 2003 marked the first time that WNF was reported along with WNND. Age, gender, date of onset of symptoms and the reporting county were the only variables in this dataset, which conforms to all HIPPA regulations. Ethical clearance was obtained from the Institutional Review Board (IRB).

Counties participating in the sentinel chicken surveillance program (Appendix VII) submit weekly serum samples taken from chickens located in various geographical sites around the state. The levels of participation among counties vary from year to year with some counties increasing the number of sites and others decrease sites. Only sera confirmed positive between the years 2001—2003 were included in this evaluation’s dataset. To evaluate temporality, each reported result date was assigned a week number provided by the Microsoft Excel formula WEEKNUM. A weekly seroconversion rate was then calculated for the chicken population using the following equation:

\[
\frac{\text{Total # of positive chicken sera by county per week}}{\text{Total # of chickens bled by county per week}}
\]
Two methods of collecting dead birds information are described in the
*Surveillance and Control of Selected Arthropod-borne Diseases in Florida 2003*; dead
bird web based reporting and laboratory testing of dead birds. The web based reporting
system uses the internet as an interface for citizens or County Health Department officials
to report dead birds. This reporting system could not be included in this dataset because
information was not available for all years included in this evaluation. Counties
participating in the dead bird surveillance program are listed in Appendix VIII. Variables
included in the dead bird dataset consisted of specimen collection date, laboratory result
and county of collection. Dead birds laboratory confirmed positive for West Nile virus
collected from 2001 through 2003 were assigned a week based on collection date
according to the WEEKNUM Microsoft Excel formula.

The mosquito dataset included species specific mosquito pool results collected by
mosquito control agencies from counties submitting to the FDOH, BOL. The counties
participating are listed in the table shown in Appendix VIII. The dataset for this study
included all laboratory confirmed positive mosquito pools collected from the year 2001
through 2003. Mosquito Control District testing was not included in the data analyzed.
The only variables used in analysis were collection date, laboratory result and county of
collection. These were queried from the FDOH, BOL’s Microsoft Access Database for
2002 and 2003; for 2001 the variables were queried from a Microsoft Excel Database.
The collection date was used to generate a week number using the Microsoft Excel
formula WEEKNUM.

Data from the various sources were excluded based on certain criteria. The data in
each surveillance system were included if the collection date, laboratory result and
county of collection were available. If one or more of these variables were missing, the data was omitted from the final dataset.

The population of each county was acquired through Florida CHARTS for the years 2001, 2002 and 2003 for the purpose of calculating incidence rates. Florida CHARTS provides an estimate of the total population and is classified by age, race and gender. Only the estimated total populations for counties with confirmed WNND or WNF reports were used.

Analysis

Descriptive and summary statistical methods were used for examining the four datasets. Microsoft Excel was used to generate graphical representations of distributions for clinical demographics, confirmed human case reports, sentinel chicken positive conversion rates, positive birds, positive mosquito pools and dead bird reports. The clinical demographics examined included age and gender. In order to adequately represent the distribution of age it was divided into four categories <18 years, 19 to 36 years, 37-54 years and >55 years.

Incidence was calculated separately for all counties with established clinical cases for each year (2001, 2002 or 2003). The calculation was computed according to the given incidence equation:

\[
\text{Incidence} = \frac{\text{Number of New Cases}}{\text{Specified Population}}
\]

The number of new cases is the total confirmed WNND and WNF case reports for the associated county by year. The specified population is the estimated population of the county where a confirmed case was reported during the indicated year. The counties were
organized by region and assigned a specific number indicating location to adjacent counties (Appendix IX). The statewide incidence was computed by adding the total new cases from each county and dividing it by the population of each county with new cases.

The temporal distribution was determined using week numbers generated from the collection date of dead birds and mosquito pools, reported date for sentinel chicken results and date for start of symptoms for clinical cases. The summary statistics calculated for each surveillance type and human cases were used to determine the starting and ending week for the West Nile transmission season. Outliers were defined as data points outside the calculated transmission season. In order to show these distributions were not due to chance alone, a moving average analysis was done, plotting the average for each surveillance type with the calculated moving average by region using Microsoft Excel. The average for each year’s surveillance systems was calculated by pooling the data by week, for each year and dividing by the total number of years observed (three).

Comparative summary statistics were generated for each surveillance system and clinical cases by Analyse-it version 1.71 a Microsoft Excel add-in program using a generated box-plot. Box-plots graphically show the central location and scatter/dispersion of the observations of the samples. Refer to figure 17 on page 43 for an example showing a box-plot. The blue line series shows parametric statistics where the blue diamond represents the mean and the confidence interval around the mean. The notched blue lines show the parametric percentile range. The notched box shows the median, lower and upper quartiles, and confidence interval around the median. The dotted-line connects the nearest observations within 1.5 IQRs (inter-quartile ranges) of the lower and upper quartiles. The red crosses (+) and circles (o) indicate possible outliers - observations more
than 1.5 IQRs (near outliers) and 3.0 IQRs (far outliers) from the quartiles (Analyse-it, 2003).

The mosquito pool and dead bird data sets were graphed using columns and lines with a primary and secondary axis (y and z) by the total number of submitted specimen and the percent positive using Microsoft Excel. The percent positive was calculated using the following equation:

\[
\text{Number of Positive per Week} = \frac{\text{Number of Positive per Week}}{\text{Total Number of Specimen Submitted per Week}}
\]

Descriptive statistics were used to represent distributions for the Florida regions (Panhandle, North, Central and South) depicting the overall arbo surveillance outcome preceding and during the time period that included clinical cases. These were accomplished by pooling county data into regions based on physiographic climate differences shown in Appendix IX (Day, 1996). The surveillance data included the number of sentinel, dead bird and mosquito positives and the total reported dead birds. These were all graphed together using columns and lines with a primary and secondary axis (y and z) where the columns represented the number of positive mosquito pools and sentinels with the number of dead bird positives were represented by the line using Microsoft Excel. The numbers of clinical cases were represented by arrows showing the week of the onset of symptoms.

Spatial analysis was accomplished using ArcView 3.3 (Environment Systems Research Institute, Inc, Redlands, CA, USA) for cartographical images. The shape file for
Florida with county boundaries was obtained from Environment Systems Research Institute, Inc (ESRI) files for the United States. Each type of surveillance (sentinel chicken, dead birds submitted, mosquito and total dead birds reported) for 2001, 2002 and 2003 were independently examined with confirmed WNND and WNF reports. A correlation using Pearson’s correlation coefficient was used to determine an association between the number of clinical cases and each surveillance type. The correlation graph and values were generated through the Microsoft Excel add-in Analyse-it.

Analytical statistic methods were performed using SAS (Statistical Software Systems v8.0, SAS Institute Inc., Cary, NC USA) for multivariate Poisson Regression (PROC GENMOD) analysis of the datasets. This regression model was used because the response variable was an incidence rate. The Poisson regression is a member of a class of generalized linear models, which is an extension of traditional linear models that allows the mean of a population to depend on a linear predictor through a nonlinear link function and allows the response probability distribution to be any member of an exponential family of distributions (Neter, 1996; Argesti, 1996; Stokes, 2000). The PROC GENMOD of SAS can fit a wide range of generalized linear models. The following SAS statements use PROC GENMOD to fit the Poisson regression:

\[
\log(\mu_i) = \log(p_i) + \beta_0 + \beta_i (\text{surveillance system})
\]

(where \(\log(p_i)\) is used as the offset in the calculations: offset=log of the population for each region by year, surveillance system=positive dead bird rate, positive mosquito pool rate or sentinel seroconversion rate)

when considering the effects of region:

\[
\log(\mu_i) = \log(p_i) + \beta_0 + \beta_i (\text{central}) + \beta_2 (\text{north}) + \beta_3 (\text{panhandle}) + \beta_4 (\text{south}) + \beta_5 (\text{surveillance system})
\]

(where \(\log(p_i)\) is used as the offset in the calculations: offset=log of the population for each region by year, \(\beta_4 (\text{south})\) was the reference region therefore=0.00, surveillance system=positive dead bird rate, positive mosquito pool rate or sentinel seroconversion rate)
A combined year analysis for 2001, 2002 and 2003 classified by region for all surveillance systems was used to identify the best model for the early detection of West Nile associated human illness. The surveillance types were set as parameters and modeled collectively and independently (due to differences in participation levels) with and without regional data. The incidence rate (calculation shown previously) which based on number of confirmed WNND and WNF the case reports was set as the response variable for the variable of sentinel chicken seroconversion rates, percent of positive dead birds, and percent of positive mosquito pools per region by week reported by the FDOH. Calculations for percent positives were done using the previously shown equation. These parameters and the response variable were analyzed independently in a combined model and by individual year (2001, 2002 and 2003) annually. There was not consistent participation in all the surveillance types by the counties therefore; a collective model of all the parameters was not used.
Results

Demographic Analysis

Gender

The graph in Figure 5 illustrates the number of cases of West Nile Infection for 2001-2003. The graph shows the number of males is approximately twice as high as the number of females with West Nile.

The graph for individual years and number of West Nile cases by gender is represented in Figure 6. This graph exhibits an overall higher trend of diagnosed males ($R^2 = 0.5535$) than females and the number of West Nile infections in males decreased from 2001 to 2002 but had a sharp increase in 2003. The number of females with West Nile disease shows a slight increase from 2001 to 2002 but remains constant from 2002 to 2003. The overall trend for females is consistent over the three years with an $R^2 = 0.75$. The regression trend line equation for males and females were $y = 9x + 10.333$ and $y = 2x + 2.6667$ respectively (male slope=9 and female slope=2).
Figure 5. **Total Number of West Nile Cases and Gender for 2001-2003.**
The number of males is much greater than the number of females infected with WNND. The magenta bar represents the number of female WN clinical cases and the blue bar represents the number of male WN clinical cases.

![Bar chart showing the total number of West Nile cases by gender for 2001-2003.](chart1.png)

Figure 6. **Cumulative Number of WN Cases for Gender by Year.**
The male cases are shown in blue, females in magenta. The black line represents the fitted regression line for the total number of cases for each gender (males and females) over all years.

![Line chart showing the cumulative number of West Nile cases by year for males and females.](chart2.png)
Age Groups

Age group distributions for 2001, 2002 and 2003 were combined and are shown in Figure 7. Among the age groups the greatest number of WNND is seen in the population over 55 with the lowest number under 18 years old. There is a perfect correlation (Pearson’s Correlation Coefficient = 1.00 with a p-value of 0.0034) between the age groups and the number of cases of severe disease. This shows that as age increases (by age group) the number of WNND cases also increases. Prior to 2003, primarily hospitalized severe cases were diagnosed by laboratory testing. Both WNND and WNF are reported during 2003. Therefore, the 2003 data was further separated by infection type: WNND and WNF. The graphical representation for specific infection type and age group indicates that WNF is more prominent among 36 to 54 year olds whereas WNND occurs more often in the older age group.

When gender and age are grouped together (Figure 9) the data further revealed the increase in the number of clinical cases as age increases regardless of gender, but the gender trend noted in Figure 5 remains constant.
Figure 7. **WNND by Age Group for 2001-2003.**
The red bars represent the number of WN clinical cases for each age group.

![Bar graph showing WNND by age group for 2001-2003.](image)

Figure 8. **Age Comparison for WNND and WNF for 2003.**
This graph shows the age groups separated by WNND (red bar) and WNF (blue bar).

![Bar graph showing age comparison for WNND and WNF for 2003.](image)
Figure 9. **WNND by Gender and Age Group for 2001-2003.**

This graph shows the number of males (blue bars) and the females (magenta bars) for all age groups with WNND.
West Nile Incidence Rates

Figures 10a through 10c depict incidence rates for counties with West Nile associated clinical cases for 2001-03. The graphs were also grouped by region and generally ordered by location from west (left) to east (right) and north (top) to south (bottom) shown in Appendix XI. The highest incidence rates were seen in Madison, Baker, and Gulf counties respectively, and the lowest reported case rates were seen in Palm Beach and Dade counties. These graphs show a clustering of WN disease by location. Comparison of counties located adjacent to one another, shows higher incidence counties tend to be located together. This pattern shows the highest incidence in the panhandle and adjacent areas; minimal activity in most of the eastern peninsular counties; moderate in the southernmost counties. Statewide incidence rates increased linearly from year to year with an $R^2 = 0.9907$ as shown in Figure 11. Appendix XI shows the raw data for the number and incidence of clinical cases in each county by year.
Figure 10a.  West Nile Incidence Rate by County, Region and Location for 2001.  
The x-axis represents the counties listed in legend by color.

Figure 10b.  West Nile Incidence Rate by County, Region and Location for 2002.  
The x-axis represents the counties listed in legend by color.
Figure 10c. West Nile Incidence Rate by County, Region and Location for 2003.
The x-axis represents the counties listed by color in legend.

Figure 11. West Nile Clinical Statewide Incidence Rate for 2001 through 2003.
This graph shows the incidence for each consecutive year represented by the blue diamonds. The black line is the fitted regression line for the three years.
Surveillance

Temporal Distribution

Figure 12 shows the peak transmission period for total number of clinical cases, which begins at week 25 and ends at week 50. The transmission period for sentinel chicken positives (Figure 13) was determined by pooling the totals of sentinel chicken positives by week for each year. The peak transmission period begins at the 24th week and ends at the 52nd week. Outliers (from week 1 through week 5) are present in the data, but were excluded from the analysis because they were probably residual positives from the previous year. The temporal graph of the number of positive dead birds (Figure 14) indicates a peak transmission period from week 24 to week 53 and mosquito pools beginning at the 20th week and ending in the 50th week (Figure 15). An overall transmission period beginning at week 20 and ending with week 53 created by combining all four sets of surveillance data. The line graphs plotted for the averages of each surveillance type for the combined years (2001-2003) with the calculated moving averages shown in Appendix XI indicates comparatively similar systematic patterns, indicating that the data’s distribution is not due to chance alone.
Figure 12. Sentinel Chicken Positive West Nile Weekly Seroconversion Rate by Week for 2001 – 2003.

Figure 13. West Nile Clinical Cases by Week for 2001—2003.
Figure 14. Total Number of West Nile Positive Dead Birds by Week for 2001 – 2003.

Figure 15. Total Number of West Nile Positive Mosquito Pools by Week for 2001 – 2003.
Clinical Cases

Figure 16 represents the number of clinical cases per week and separated by year. The data reveals a different timing of peak incidence of cases each year. The number of cases does not show consistent a pattern but occur sporadically within the transmission season from year to year. However, the cumulative weeks for the individual years show a distribution similar to a bell shaped curve. During 2001, the highest weekly number of confirmed human cases of WNV was two, occurring in weeks 28, 36 and 40. In 2002 a high of four cases occurred at weeks 39 and 44. Eleven cases was the highest number of weekly clinical cases, and occurred during weeks 35, 38 and 39 during 2003. The total cases of West Nile Disease in humans in 2001 there were 12, in 2002 there were 35 and in 2003 there were 92.

The overall distribution of the number of diagnosed cases is shown as a box plot in Figure 17. For these samples the box plot demonstrates an increase in both the average and total numbers of clinical cases by week from 2001 to 2003. Far outliers are seen in 2001 and 2003 with near outliers observed in each year.
Figure 16. Number of Clinical Cases of West Nile per Week during Peak Transmission Season.

Figure 17. Box-plot for the Average and Total Number of Clinical Cases for 2001, 2002 and 2003.

This graph shows a distinct increase from 2001 to 2003 in the average and total numbers of cases by week per year.
**Sentinel Chicken Surveillance**

There were a total of 204 sentinel sites with a total of 2,128 sentinel chickens monitored during 2001 of which 202 seroconverted for WN antibodies (Stark, 2001). In 2002 there were a total of 202 sentinel sites with 1,093 WN seroconversions out of a total of 3,356 sentinel chickens (Stark, 2002). There were 289 sentinel sites with 4,361 total chickens monitored showing 1,343 WN positive seroconversions during 2003 (Stark, 2003).

The graph depicted in figure 18 for sentinel chicken serosurveillance has several different peak weeks for each year. The highest seroconversion rate for 2001, seen at week 46, was 0.0235 and was significantly lower than observed in the following two years. The highest rate for 2002 was 0.0822 at week 44, with other weeks of notably higher rates at weeks 38, 39, 41 and 43. In 2003 the maximum seroconversion rate was 0.1127 during week 47, and comparably high rates by week also occurred at weeks 33, 34, 35, 37 and 38.

The overall distribution of the seroconversion rates of the sentinel chicken surveillance data by week is shown in the box plot (figure 19). This illustrates an increase in the average and total rates of seroconversion for each consecutive year from 2001 to 2003. There are near outlier observation for the seroconversion rate in 2001 and 2003 with far outliers in 2001 only. There were no observed outliers in 2002.
Figure 18. Sentinel Chicken Surveillance Rate during Transmission Season for 2001 - 2003.

This graph shows a distinct increase from 2001 to 2003 in the average and total seroconversion rate per year.


This graph shows a distinct increase from 2001 to 2003 in the average and total seroconversion rate per year.
**Avian Surveillance**

There were a total of 7,675 total dead avian samples submitted with 1,106 testing positive for WNV (Stark, 2001). During 2002 there were a total of 4,020 of which 439 tested positive for WNV (Stark, 2002). There were 2,320 dead birds submitted during 2003 with 486 testing positive for WNV (Stark, 2003).

Figure 20a, statewide total for positive dead avian specimen, shows some consistent curves for the peak period during 2001, 2002 and 2003. The largest numbers of cases occurred from weeks 32 to 42 each year. The highest number of dead bird positives in a week for 2001 was 126 which was greater than the numbers observed in 2002 and 2003. The greatest number of positives for 2002 was 36, at week 32, other notably high numbers were seen during weeks 41 and 35. In 2003 the greatest number of positives was 52, observed during week 32, with high numbers also occurring from week 33 to week 35.

Figure 20b shows the observed percent of positive dead avian specimen out of the total number submitted. The highest rate observed for the all the weeks in 2001, 2002 and 2003 was week 24 in 2001 which was 100% positive out of the submitted birds (1 submitted and 1 positive). The overall rates were highest in 2003 with the highest rate at 0.6 during week 36. The 2001 and 2002 rates had similar observed rates with the highest in 2001 (excluding week 23) 0.25 during week 52 and 0.28 during week 29.

Figures 21 a, b and c show the total number of birds submitted with percent of positive samples for each year (2001, 2002 and 2003). In 2001 (figure 21a), the percent increases variably with an increase in total birds submitted except for the first positive (week 24). During 2002 shown in figure 21b the graph shows sporadic increases in the
percent positive however, the overall increase is greater during weeks with a larger number of submissions. Excluding the first observation in 2001 the percent positives from 2001 and 2002 have similar overall patterns with highest percents at 19% and 22% respectively. The graph for 2003 (figure 21c) shows a more consistent relationship with the observed temporal distribution and exhibits increased percent positives with amplified dead bird submitting. All three years had a significant positive correlation between the percent of positive dead birds and the total number of submitted (p-value < 0.0001). The specific Pearson’s correlation coefficient for each year was 0.79 for 2001, 0.70 for 2002 and 0.76 for 2003.

The box plot shown in Figure 22 for the numbers of positive dead birds does not follow the previous distributions seen with clinical cases and sentinel chicken serosurveillance. The box plot shows that average number of positive dead birds by week decrease from 2001 to 2002 and increase from 2002 to 2003. The total number of positives indicated by the plots follow the same trend however, near outliers are seen in 2001 and 2002 with far outliers observed in 2001. There were not outlier observations in 2003.

The total submitted dead avian specimen shown in figure 23 for the entire year indicates the submissions of dead birds begin to increase at approximately week 24. The highest numbers of submissions for all three years were observed between week 31 and 41. The highest total submitted dead birds for the three years were 732 in 2001, 254 in 2002 and 117 in 2003.

The box plot in figure 24 for total submitted dead birds decreased from 2001 to 2003. The overall numbers submitted are significantly higher in 2001 and decline
dramatically over 2002 and 2003. For the total dead birds submitted the near outliers are seen in all three years with one far outlier observation for 2002.
Figure 20a.  Total Number of West Nile Positive Dead Birds during Transmission Season for 2001 – 2003.
This graph shows the highest numbers of positives during 2001. The number of positives per week was higher in 2003 compared to 2002.

Figure 20b.  Dead Birds Percent Positive.
Percent positive was calculated by dividing the number of WN positive dead birds by the total number submitted.
Figure: 21a. The Total Dead Birds Submitted and the Percent of Positive Dead Birds by Week for 2001.
Percent positive was calculated by dividing the number of WN positive dead birds by the total number submitted.

Figure: 21b. The Total Dead Birds Submitted and the Percent of Positive Dead Birds by Week for 2002.
Percent positive was calculated by dividing the number of WN positive dead birds by the total number submitted.
Figure 21c. The Total Dead Birds Submitted and the Percent of Positive Dead Birds by Week for 2003.
Percent positive was calculated by dividing the number of WN positive dead birds by the total number submitted.

![Graph showing total dead birds submitted and percent positive by week for 2003.]

Figure 22. Box-plot the Number of Positive Dead Birds for 2001, 2002 and 2003.

![Box-plot showing number of positive dead birds for 2001, 2002, and 2003.]
Figure 23.  Total Dead Birds Submitted for 2001 – 2003.

Figure 24.  Box-plot for Total Submitted Dead Birds for 2001, 2002 and 2003.
Mosquito Surveillance

In 2001 there were 1,378 submitted mosquito pools of which ten tested positive for WNV (Stark, 2001). The total number of mosquito pools submitted in 2002 was 3,886 with 25 testing positive for WNV (Stark, 2002). There were 6,292 mosquito pools submitted during 2003 with 42 positive for WNV (Stark, 2003).

The histogram pictured in Figure 25 for positive mosquito pools has the lowest number of positives among all the surveillance systems. The collective distribution for 2001, 2002 and 2003 is similar with the curves showing a range for the peak positive activity from week 29 to week 35. The highest number of mosquito pool positives for 2001 seen at week 39 was two. The highest number of positives for 2002 was four at weeks 31 and 32 with three positives during 29 and 35. The data for 2003 was notably greater than the previous years with 18 total positives at week 32 and high numbers of positives during week 31 and 33.

Figure 26 shows the observed percent of positive mosquito pools out of the total number of mosquito pools submitted for 2001-2003. The highest percent positives observed during 2001 was 6.7% at week 40, 2002 was 3.5% at week 43 and 2003 was 6.5% at week 32. The overall rates were higher in 2001 and 2003. The overall distribution for the years observed were sporadic, exhibiting no consistent patterns.

Figures 28 a, b and c show the total number of mosquito pools submitted with the percent positive for each year (2001, 2002 and 2003). The graph for 2001 (figure 28a) shows that the percent appears sporadic indicating that there is no correlation with number submitted (Pearson’s Correlation Coefficient=0.31 and p-value=0.097). The 2002 distribution of percent positive mosquito pools and total submitted shown in figure 28b
has the best correlation (Pearson’s Correlation Coefficient=0.37 and p-value=0.0087) of the three years. The 2003 distribution has an overall higher number submitted mosquito pools compared to the previous two years. Pearson’s correlation coefficient for 2003 was 0.38 with a significant p-value of 0.0415. The highest number of submitted mosquito pools was 181 during weeks 28 and 46 in 2001. The highest in 2002 was 268 during week 32 and 221 during week 25 in 2003. The overall data for mosquito pool positives shown in the box plot (figure 27) illustrates an increase in the average and total numbers of positives detected per week for 2001, 2002 and 2003. There were near outlier observations in 2002 and 2003 with far outliers in 2001 and 2003.
Figure 25. **Total Positive Mosquito Pools during Transmission Season for 2001 – 2003**

![Graph showing total positive mosquito pools during transmission season for 2001 - 2003.]

Figure 26. **Percent of Positive Mosquito Pools during Transmission Season for 2001 – 2003.**

![Graph showing percent positive mosquito pools during transmission season for 2001 - 2003.]

Figure 27. Box-plot for Total Positive Mosquito Pools for 2001, 2002 and 2003.

Figure 28a. The Total Positive Mosquito Pools and the Percent of Positive Mosquito Pools by Week for 2001.
Percent positive was calculated by dividing the number of WN positive mosquito pools by the total number submitted.
Figure : 28b. The Total Positive Mosquito Pools and the Percent of Positive Mosquito Pools by Week for 2002.
Percent positive was calculated by dividing the number of WN positive mosquito pools by the total number submitted.

Figure : 28c. The Total Positive Mosquito Pools and the Percent of Positive Mosquito Pools by Week for 2003.
Percent positive was calculated by dividing the number of WN positive mosquito pools by the total number submitted.
Regional Surveillance

Panhandle Region

To show results within similar physiographic climates, Florida was divided into four geographic regions: Panhandle, Northern, Central and Southern regions. Figures 29a, 29b and 29c correspond to the Panhandle Region, while Figures 30-32a, b and c correspond to the Northern, Central and Southern regions respectively. The combined year average and the moving average plots for each region are shown in Appendix XI. The graph for each region (Panhandle, North, Central and South) and average positive outcome pooled for 2001-2003 for Humans, Dead Birds, Mosquito Pools and Sentinel Chickens by week each show similar patterns compared to the calculated moving average. This relationship would indicate that the distributions seen within each region for the surveillance types are most likely not due to chance.

Figure 29a depicts positive mosquito pools, dead birds and sentinels for the Panhandle region during 2001 with human cases pin-pointed. There were two clusters of human cases. Positive dead birds and mosquito pool first occurred about four weeks prior to the first human case and the first increase in positive sentinel chickens was seen two weeks prior to the first case. A second peak in positive mosquito pools and dead birds occurred two to three weeks prior to the second cluster of human cases. Nevertheless, in the panhandle, the peak for positive sentinels began approximately one week after the last confirmed human case.

Figure 29b shows the regional graph of the 2002 Panhandle region indicates two clusters of human cases approximately seven weeks apart, each with corresponding
increases in the number of sentinel and dead bird positives before the initial human case occurred. However, the mosquito surveillance program did not detect any positives for this region during 2002. This is consistent with the current mosquito trends where there has been little or no detection for West Nile from submitted samples.

Figures 29c illustrates similar positive distributions in the dead bird and sentinel chicken flock populations in the panhandle region for 2003. There were a few mosquito pool positives detected, but these numbers are small (week 28 had 1 positive pool and week 32 had 13 positive pools) and did not correspond to the distribution trends. There were a large number of clinical cases (31) spread throughout the entire transmission period making individual analysis of separate peaks within the period impossible. The graph also shows a lower total number of dead bird positives for the surveillance systems than the previous years, but a much higher total for the number of sentinel positive. The first clinical WN case occurred one week prior to the detection of virus or antibodies in sentinels, dead birds or mosquitoes. With the exception of mosquitoes, the virus was detected at an increasing rate within the weeks following the first clinical case; however, dead birds had a bimodal distribution with a decrease in the number positive midway through the transmission season.
Figure 29a. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Panhandle Region during 2001.
The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.

Figure 29b. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Panhandle Region during 2002.
The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.
Figure 29c. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Panhandle Region during 2003.

The two axes, y (number of positive mosquito pools) and z(number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.
Northern Region

The Northern region of Florida for the year 2001 (Figure 30a) shows a corresponding increase in the rate of West Nile positive sentinels and the number dead bird with the first three (of four) clinical cases. There was one case detected after the sentinel and dead bird positives peaked. There were no positive mosquito pools for this region during 2001.

In 2002 (Figure 30b), there were positive sentinel outcomes before the first human case and numbers continued to increase throughout the transmission period with three peaks each approximately one week prior to each of the clusters of human cases. Dead bird positives were detected in low numbers before the first human case with peaks prior to or during the separate clusters of human cases. Mosquito pools provided an early indication of human cases for this period as well, peaking approximately two weeks after the first clinical case and four weeks prior to the second case.

The graph for the northern region during 2003 (Figure 30c) shows an increase in the number of confirmed human cases. Therefore, specific peak evaluation for the surveillance systems within the time period for human transmission was not possible. Nevertheless, positive dead birds and sentinels were observed before the initial human case, and increased and decreased with the occurrence of clinical cases. No mosquito pools were positive during this period for northern Florida.
Figure 30a. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Northern Region during 2001.
The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each.

![Graph showing the number of positive avian, sentinel, and mosquito cases in 2001.](image)

Figure 30b. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Northern Region during 2002.
The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each.

![Graph showing the number of positive avian, sentinel, and mosquito cases in 2002.](image)
Figure 30c. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Northern Region during 2003.

The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.
Central Region

The distribution of the data from 2001 for the central Florida region figure 31a demonstrates an increase simultaneously with and following the first and only clinical case diagnosed. Positive dead birds were detected the same week as the human case and increased after. One sentinel positive was observed three weeks after the clinical case. There were two positive mosquito pools detected 15 weeks after the human case.

Evaluation of the graph for the central region in 2002 (figure 31b) shows a different picture than 2001. There was a spike in the number of positive mosquito pools and with an increase in the number of positives among the sentinel population approximately eight weeks before the first human case was diagnosed. There was a small increase in positive dead birds four week before the first case. All three surveillance systems showed a spike in numbers four to eight weeks before the second cluster of clinical cases. However, mosquito pools had only one positive result. The number of dead bird positives peaked five weeks before the start of the second group of cases whereas the sentinels had a significant elevation at week 41, a week before the next cluster of clinical cases.

Figure 31c for the 2003 central Florida region shows an increase in the number of positive sentinel chickens starting four weeks before the first human case and positives began tapering off after the second case and began increasing again before the third human case. The numbers of dead bird positives were extremely low for this year directly corresponding with the overall numbers of birds submitted for 2003. Even with the small numbers, there were positives detected before the first clinical case. Two mosquito pools were positives, one and two weeks before the last human case.
Figure 31a.  The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Central region during 2001.
The two axes, y (number of positive mosquito pools) and z(number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.

Figure 31b.  The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Central Region during 2002.
The two axes, y (number of positive mosquito pools) and z(number of positive dead avian) have different maximums, minimums and scales for each.
Figure 31c. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Central Region during 2003.

The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.
**Southern Region**

The graph for the southern region of Florida in 2001 is shown in figure 32a. This shows the initial clinical case before any surveillance positives were detected. However, two weeks prior to the second case positive WN birds were detected and one week prior WN was detected in mosquito pools. Sentinels showed an increase in WN antibody detection nine weeks before the second human case.

The distribution for Florida’s southern region in 2002 is illustrated in figure 32b showed a small increase in positive sentinels and dead birds before the first human case was diagnosed. An increased detection of West Nile was seen in sentinel and dead bird positives two to three weeks before the second and third cases were seen. There were two mosquito pool positives detected four weeks before the third case.

Figure 32c, the graph of the southern region for 2003, shows a distribution similar to the previous two years. There were a few positive sentinels and three positive dead birds detected before the first case. They both increased over a period coinciding with diagnosed cases and decreased after the last clinical case. There were a few mosquito pools that tested positive around the same week of the last human case and ten weeks after the last case.
Figure 32a. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Southern Region during 2001. The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each.

Figure 32b. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Southern Region during 2002. The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each.
Figure 32c. The Number of Positive Avian, Sentinels and Mosquitoes with human cases for the Southern Region during 2002.
The two axes, y (number of positive mosquito pools) and z (number of positive dead avian) have different maximums, minimums and scales for each. The breaks in the lines for positive avian represent no specimen submitted for that week.
**Clinical Cases by Region**

The number of clinical cases by region figure shows the epicenter in the northern region for 2001 and 2002 and the panhandle region for 2003. The graph also shows the central region had the least number of cases in 2001 and 2003 with lowest number of cases for 2002 in the southern region. All regions showed an increase in number of human cases each year, with the exception of the central region which had a decrease in clinical cases for 2003.
Figure 33. **Number of Clinical Cases by Region for 2001-2003.**
Regions are organized north to south and east to west. Each year is indicated in the legend by a different color.
Spatial Analysis

Positive Dead Birds and Clinical Cases

The overall trend for dead bird positives appears to coincide with the number of clinical cases diagnosed per county. The counties that showed the highest number of positive dead birds also had the highest number of clinical cases. Figure 34a shows the highest total number of clinical cases in Escambia and Bay counties as 19 and 14 respectively. These counties also had the highest range of positive dead birds between 120-206 total. Other counties of interest for the collective year spatial analysis includes Duval, Dade, Santa Rosa, Okaloosa and Marion which all have higher numbers of clinical cases and positive dead birds than the other counties observed. The correlation plot (figure 34b) shows a significant positive correlation between the number of clinical cases and the number of positive dead birds by county for the three years (Pearson’s correlation coefficient=0.76 and p-value<0.0001). There were apparent outliers in the scatter plot which may have influenced the fit of the line. A scatter plot excluding these observations is shown in figure 34c. The outlier observations were determined by a box-plot which indicated two separate weeks with the highest number of clinical cases numbers (14 and 19). The scatter plot showed less of a positive correlation then what was seen in the plot including the outlier data.

Figures 35a, 36a, and 37a show the dead bird distribution and diagnosed clinical cases by county for 2001, 2002 and 2003 whereas figures 35b, 36b and 37b show the scatter plot correlation between the number of positive dead birds and clinical cases by county for each year. In 2001 (Figure 35a) there were insufficient number of clinical cases to accurately evaluate the correlations (figure 35b) with positive dead birds. The
Pearson’s coefficient correlation was 0.23 with a p-value of 0.0651. However, 2002 and 2003 show similar trends to the combined year map and correlation. Box-plot analysis did not indicate any outlier observations.

In 2002 depicted in Figure 36a there are two counties (Escambia and Marion) which show a correlation between the numbers of positive dead birds and the number of clinical cases. Escambia County had the highest numbers of human cases (seven) and positive dead birds (in the range from 35-121). Marion County had the second highest number of human cases but shared the range for positive dead avian specimen (20-35) with several other counties that had fewer clinical cases. The Pearson’s correlation coefficient was 0.92 with a p-value of < 0.0001 showing that there is a significant positive correlation with the increase in the number of positive dead birds with an increase in the number of clinical cases. Outliers were present in the data determined by box-plot analysis. These observations were at three different weeks specifically excluded was the data points that had seven clinical cases with 121 positive dead birds and three clinical cases with 34 positive dead birds. The scatter plot excluding these observations is shown in figure 36c. The scatter plot showed a positive correlation however, it was lower then the scatter plot including the outliers.

The map for 2003 shown in figure 37a has four counties which showed corresponding increases with the range of numbers of positive dead birds and human cases. Figure 37b indicates there is a significant correlation between the number positive dead birds and diagnosed clinical cases (Pearson’s correlation coefficient= 0.91 and p-value < 0.0001). The highest counties for clinical cases are Bay and Escambia with 14 and 12 clinical cases however, these were considered outlier observations in the box-plot
calculations. The scatter plot excluding these data are shown in Figure 37c which indicates a smaller positive correlation between clinical cases and positive dead birds. These two counties also had the most positive dead birds ranging from 46-103. This corresponds to the total combined year data. Okaloosa and Dade counties had eight and six clinical cases respectively with the total number of dead birds ranging between 17 and 45. Broward County also had corresponding numbers with number of positive dead avian specimen (ranging from 3-5) and clinical cases (four). Several counties had one, two or zero human cases where the total number of positive dead birds ranged from 0-2. Gulf, Lee and Lafayette counties submitted no dead birds but reported clinical cases.
Figure 34a. Florida West Nile Cumulative Positive Dead Bird Distribution and Clinical Cases by County for 2001-2003.
Intensity of color indicates numbers of West Nile positive birds detected. The areas designated without color signify counties that did not submit any dead birds for testing.
Figure 34b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for the Pooled Years 2001-2003. The black line represents the fitted regression line for the number of clinical cases and positive dead avian.

Figure 34c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for the Pooled Years 2001-2003 excluding outlier observations. The black line represents the fitted regression line for the number of clinical cases and positive dead avian.
Figure 35a. Florida West Nile Positive Dead Bird Distribution and Clinical Cases by County for 2001.
Intensity of color indicates numbers of West Nile positive birds detected. The areas designated without color signify counties that did not submit any dead birds for testing.
Figure 35b.  Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2001.
The black line represents the fitted regression line for the number of clinical cases and positive dead avian.
Figure 36a. Florida West Nile Positive Dead Bird Distribution and Clinical Cases by County for 2002.
Intensity of color indicates numbers of West Nile positive birds detected. The areas designated without color signify counties that did not submit any dead birds for testing.
Figure 36b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2002. 
The black line represents the fitted regression line for the number of clinical cases and positive dead avian.

Figure 36c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2002 excluding outlier observations. 
The black line represents the fitted regression line for the number of clinical cases and positive dead avian.
Figure 37a. Florida West Nile Positive Dead Avian Distribution and Clinical Cases by County for 2003.
Intensity of color indicates numbers of West Nile positive birds detected. The areas designated without color signify counties that did not submit any dead birds for testing.
Figure 37b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2003.
The black line represents the fitted regression line for the number of clinical cases and positive dead avian.

Figure 37c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Dead Birds by Week for 2003 excluding outlier observations.
The black line represents the fitted regression line for the number of clinical cases and positive dead avian.
**Positive Mosquito Pools and Clinical Cases**

Mosquito surveillance was performed in 23 counties during 2001—2003. The overall distribution of positive mosquito pools did not correlate with the number of clinical cases. However, there were some significant associations for a few individual counties. The counties participating in the state mosquito surveillance program are listed in the table found in Appendix VIII.

The combined year data for West Nile positive mosquito pools (figure 38a) showed two counties (Escambia and Gulf) with positive mosquito pools ranging from 1 to 3 that also had clinical cases. However, twelve out of the 23 participating counties had no WNV positive mosquito pools, though some of these counties (Lee, Dade and Gulf) had more than four human cases each. Several counties including St. Johns, Volusia, Pinellas, Collier, Monroe and Dade also had high levels of positive mosquito pools (18-32) with a low number of clinical cases (less than four). The correlation scatter plot (figure 38b) for the combined years showed an inverse association between the number of positive mosquito pools and the number of clinical cases (Pearson’s correlation coefficient = -0.17 with a p-value = 0.4399). This indicates that there is no linear relationship with the number of positive mosquito pools and clinical cases by county for the combined years. The outliers determined by the box-plots were the observations with 11, 14 and 19 clinical cases. The scatter plot shown figure 38c for the cumulative years excluding these outliers shows a similar negative correlation as compared to 38b.

The individual year maps for 2001, 2002 and 2003 positive mosquito pool distribution and diagnosed clinical cases by county are shown in figures 39a, 40a and 41a. During 2001 (Figure 39a) there were not enough clinical cases to accurately evaluate
the possible trends between clinical cases and positive mosquito pools. The scatter plot (figure 39b) shows a positive correlation however, it is not significant (Pearson’s correlation coefficient = 0.49, p-value=0.0908). The outliers determined by the box-plots were the observations with two clinical cases. The scatter plot (figure 39c) for the cumulative years excluding these outliers shows a negative correlation as opposed to the positive trend seen in figure 39b.

The distribution between West Nile positive mosquito pools and clinical cases (figure 40a) does not appear to be associated in 2002. This is also shown in Figure 40b with correlation graph between positive mosquitoes and clinical cases indicating a negative slope trend line. The Pearson’s correlation coefficient is -0.50 with a p-value of 0.6667 therefore, no significant correlation is seen between number of positive mosquito pools and clinical cases. This means the numbers of clinical cases from the participating counties does not correspond with the number of positive mosquito pools. The outliers for 2002 were the observation with seven clinical cases (calculated by box-plot). The scatter plot (figure 40c) is a better fit for the data then the plot including the outliers however, there is still a negative correlation.

Figure 41a shows an association in Escambia County for 2003 West Nile positive mosquito pool distribution and number of clinical cases. The specific numbers for Escambia County were 12 diagnosed clinical cases with three positive mosquito pools. Although the overall correlation shown in figure 41b is not significant (p-value=.8593 for the combined counties) there are a greater number of positive mosquitoes with a greater number of clinical cases for Escambia County. There were 19 participating counties out of which only four had positive mosquito pools. The outliers calculated by the box-plot
for 2003 clinical cases and mosquito positives were observations with 10 and 12 clinical cases. The scatter plot excluding these observations is shown in figure 41c which showed a similar positive correlation to the plot including the outliers (figure 41b).
Figure 38a. Florida West Nile Positive Mosquito Pool Distribution and Clinical Cases by County for 2001-2003.
Intensity of color indicates numbers of West Nile positive mosquito pools detected. The areas designated without color signify counties that did not submit any mosquito pools for testing.
Figure 38b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for the Pooled Years 2001 - 2003. The black line represents the fitted regression line for the number of clinical cases and positive dead avian.

Figure 38c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for the Pooled Years 2001 -2003 excluding outlier observations. The black line represents the fitted regression line for the number of clinical cases and positive dead avian.
Figure 39a. Florida West Nile Mosquito Pool Distribution and Clinical Cases by County for 2001.

Intensity of color indicates numbers of West Nile positive mosquito pools detected. The areas designated without color signify counties that did not submit any mosquito pools for testing.
Figure 39b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2001. The black line represents the fitted regression line for the number of clinical cases and positive mosquito pools.

Figure 39c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2001 excluding outlier observations. The black line represents the fitted regression line for the number of clinical cases and positive mosquito pools.
Figure 40a. Florida West Nile Mosquito Pool Distribution and Clinical Cases by County for 2002. Intensity of color indicates numbers of West Nile positive mosquito pools detected. The areas designated without color signify counties that did not submit any mosquito pools for testing.
Figure 40b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2002. The black line represents the fitted regression line for the number of clinical cases and positive mosquito pools.

Figure 40c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2002 excluding outlier observations. The black line represents the fitted regression line for the number of clinical cases and positive mosquito pools.
Figure 41a: Florida West Nile Mosquito Pool Distribution and Clinical Cases by County for 2003.
Intensity of color indicates numbers of West Nile positive mosquito pools detected. The areas designated without color signify counties that did not submit any mosquito pools for testing.
Figure 41b. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2003. The black line represents the fitted regression line for the number of clinical cases and positive mosquito pools.

Figure 41c. Scatter Plot for Correlation between the Number of Clinical Cases and Positive Mosquito Pools by Week for 2003 excluding outlier observations. The black line represents the fitted regression line for the number of clinical cases and positive mosquito pools.
Sentinel Chicken Seroconversion Rates and Clinical Cases

The cartographical representation of the serosurveillance positive seroconversion rates for the sentinel chickens and observed clinical cases for the participating counties during 2001—2003 is shown in Figure 42a. There were six counties that showed a greater number of clinical cases with higher sentinel chicken seroconversions to West Nile antibody positive. This distribution was present in Bay, Duval, Collier, Lee and Marion with the most clinical cases in Bay County. Notably, each of these counties had a seroconversion rate between 0.043 and 0.062 for the overall three years. The combined year correlation scatter plot (figure 42b) showed no significant association between the sentinel seroconversion rate and the number of clinical cases with a Pearson’s correlation coefficient of -0.09 and a p-value of 0.5891. Figure 42c shows the cumulative year scatter plot excluding outliers (determined by box-plot to be observations with 8, 11 or 14 clinical cases). The data showed a negative correlation similar to 42b which included the outliers.

The maps for 2001, 2002 and 2003 sentinel chicken seroconversion rates and diagnosed clinical cases are shown in figures 43a, 44a and 45a. The 2001 map shows (Figure 43a) similar patterns to the previous 2001 maps for mosquitoes and bird surveillance. However, there were not enough human cases to accurately evaluate the possible trends between clinical cases and sentinel seroconversion rates. The scatter plot (figure 43b) for 2001 data by week shows no correlation with a Pearson’s correlation coefficient equal to 0.21 and a p-value of 0.2648.

Figure 43a shows the distribution of the annual sentinel seroconversion rate and clinical cases for 2002 by county. The individual counties with the highest numbers of
clinical cases, Escambia and Marion County, were not participating in the sentinel program in 2002. The counties participating in the sentinel surveillance program in 2002 had low numbers of clinical cases (1 or 2); subsequently no conclusions on trends could be drawn from this map. The overall correlation (figure 43b) shows no significant association (Pearson’s correlation coefficient $r = 0.12$ and $p$-value = 0.7046).

Figure 45a, the geographical distribution of the data for the annual West Nile sentinel chicken seroconversions and positive WNV human cases during 2003, showed a corresponding increase in the observed seroconversion rates and number of clinical cases in a three of the participating counties: Bay, Lee and Duval. Bay County had the most clinical cases, 14, with a sentinel chicken seroconversion of 0.0559. Lee County had only three clinical cases but had sentinel seroconversion rate of 0.0813 which fell in the highest rate range (0.062 to 0.125) shown on the map. There were six diagnosed clinical cases in Duval County, with a seroconversion rate of 0.0478, which was in the same sentinel seroconversion rate range as Bay County. The other counties either did not participate in sentinel surveillance or they did not have considerable trends. The 2003 scatter plot (figure 45b) shows no correlation between the number of clinical cases and the sentinel seroconversion rate (Pearson’s correlation coefficient $r = 0.22$ and $p$-value = 0.5637). Figure 45c shows the scatter plot excluding the observations with 14 clinical cases (outlier). This plot shows a negative correlation as opposed to the correlation shown in figure 45b including the outlier observations which had no correlation between sentinel seroconversion rates and clinical cases.
Figure 42a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2001-2003.

Intensity of color indicates the rate of West Nile serconversion. The areas designated without color signify counties that did not submit any sentinel chicken sera for testing.
Figure 42b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for the Pooled Years 2001-2003. The black line represents the fitted regression line for the number of clinical cases and sentinel seroconversion rate.

Figure 42c. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for the Pooled Years 2001-2003 excluding outlier observations. The black line represents the fitted regression line for the number of clinical cases and sentinel seroconversion rate.
Figure 43a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2001.
Intensity of color indicates the rate of West Nile serconversion. The areas designated without color signify counties that did not submit any sentinel chicken sera for testing.
Figure 43b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2001.
The black line represents the fitted regression line for the number of clinical cases and sentinel seroconversion rate.
Figure 44a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2002.

Intensity of color indicates the rate of West Nile serconversion. The areas designated without color signify counties that did not submit any sentinel chicken sera for testing.
Figure 44b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2002. The black line represents the fitted regression line for the number of clinical cases and sentinel seroconversion rate.
Figure 45a. Florida West Nile Average Sentinel Chicken Seroconversion Rate Distribution and Clinical Cases by County for 2003.

Intensity of color indicates the rate of West Nile serconversion. The areas designated without color signify counties that did not submit any sentinel chicken sera for testing.
Figure 45b. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2003.
The black line represents the fitted regression line for the number of clinical cases and sentinel seroconversion rate.

Figure 45c. Scatter Plot for Correlation between the Number of Clinical Cases and Sentinel Seroconversion Rates by Week for 2003 excluding outlier observations.
The black line represents the fitted regression line for the number of clinical cases and sentinel seroconversion rate.
**Multivariate Poisson Regression Model**

The multivariate and univariate Poisson distribution regression model results are summarized in Appendix XII. The level of significance for all models was set at $\alpha=0.10$. The data were divided by region and week and annual (weeks 1-52) data was used for analysis on the cumulative and individual years. The response variable was set as the incidence of human cases and the explanatory variables were the rate of positives for each surveillance system with and without region in the model. Region was based on four levels: panhandle, north, central and south.

The annual individual year multivariate Poisson distribution for the incidence of clinical cases for 2001 – 2003 was separately calculated for each surveillance type considering region and not considering region in the model. For 2001 and 2002 there were no significant p-values for both models with or without region. Therefore, none of the surveillance systems for these years were good predictors for clinical cases. The values from this analysis can be found in Appendix XII. The overall model equation for all Poisson calculations for the cumulative years was:

$$\log(\mu_i) = \log(p_i) + \beta_0 + \beta_1 \text{ (surveillance system)} + t \text{ (year)}$$

$$\log(\mu_i) = \log(p_i) + \beta_0 + \beta_1 \text{ (central)} + \beta_2 \text{ (north)} + \beta_3 \text{ (panhandle)} + \beta_4 \text{ (south, reference region)} + \beta_5 \text{ (surveillance system)} + t \text{ (year)}$$

The specific $\beta$ value estimates are listed in Appendix XII.

The overall model for the pooled years 2001, 2002 and 2003 indicated each of the surveillance types (dead birds, mosquitoes and sentinels) included in separate models or together predicted clinical cases with significant p-values ($p < 0.10$). The parameter estimates for all variables are shown in Appendix XII.
The Poisson distribution for 2003 showed that positive dead birds and sentinels were the best predictors whereas mosquitoes were insignificant as predictors (values in Appendix XII). The model for the positive avian rate had a p-value of 0.0002. The predictive model equation for clinical case incidence and avian positives is \[ \log(\mu) = -8.9099 + 6.5038X_1 \] (where \( \log(\mu) \) = incidence of clinical cases, \( X_1 \) = avian positive rate). The clinical case incidence and sentinel rate model had a p-value of 0.0117 and the equation was \[ \log(\mu) = -7.9687 + 12.1318X_1 \] (where \( \log(\mu) \) = incidence of clinical cases, \( X_1 \) = sentinel positive rate). Table 1 lists the significant p-values and equations for these models.

The adjusted model including all surveillance types (dead birds, sentinels, and mosquitoes) was shown to be significant predictor during 2003. No other models for the individual years (2001 and 2002) had significant p-values. The model equation for 2003 for all surveillance types is:

\[ \log(\mu) = -8.9078 - 6.0103X_1 + 10.4488X_2 - 13.0034X_3 \]

(\( \log(\mu) \) = incidence of clinical cases, \( X_1 \) = avian positive rate, \( X_2 \) = mosquito positive rate, \( X_3 \) = sentinel seroconversion rate).

Sentinel rate was the only individual significant predictor for 2001 when the effect of region was considered. All other models for 2001 and 2002 were not considered good predictors (\( p > 0.10 \)) for human cases.

The multivariate Poisson regression analysis for the annual model for 2003 including region indicated that it had an effect with all the surveillance type rates. The panhandle region was significant for all three surveillance types. The p-values for sentinel and dead avian rates were 0.1257 and 0.016 respectively. These values were higher then the p-values without region included, where sentinels were significant in the
previous model but not in this model. The panhandle p-value for the sentinel rate model was 0.0350 and 0.0217 for the avian positive rate model. The model equation for sentinel positives is not given since it was insignificant, the values can be found in Appendix XII. The equation for clinical case incidence and positive avian rates with region was

$$\log \mu = -10.0786 + -1.4089X_1 + 0.2240X_2 + 3.4915X_3 + 0.000X_4 + 4.9847X_5$$

($$\log \mu$$=incidence of clinical cases, $$X_1$$=central region, $$X_2$$=northern region, $$X_3$$=panhandle region $$X_4$$=southern region $$X_5$$=avian positive rate). Mosquito pool rates had a lower p-value (0.2860) with region in the model where the p-value for the panhandle was 0.0217 compared to the other regions. No equation model is given because the mosquito positive rate was not significant, the specific values are given in Appendix XII and table 2 lists the significant model p-values and equations.
Discussion

The frequency of diagnosed West Nile Infections in Florida is currently low; however, the risk of future epidemics remains unknown. Research has shown that approximately 80% of West Nile infections in the areas it is present are unreported because of the mild symptoms or asymptomatic infections (CDC, 2001a). Florida’s age demographic, which includes a sizeable population over 50 years of age and warm climate, may stimulate an increase in the number of confirmed WNND and WNF cases in the future. Since the morbidity and mortality of West Nile infection increases when neurological symptoms are present, early detection of WNND is an important prevention strategy to help reduce transmission.

Demographic Analysis

Based on gender demographic analysis, men were twice as likely as women to be diagnosed with WNND or WNF during the data collection period. There has not been documented association between gender and West Nile disease. Therefore, it is possible since surveillance data for this population is very limited (3 years), the observed data trend may be due to chance. Further surveillance data will be needed to confirm whether men are, in fact, more likely to be diagnosed with WNND or WNF. If this trend continues, compounding risk factors such as a greater likelihood of men participating in outdoor activities during transmission times (i.e. dusk and dawn) and a lower compliance with personal mosquito protection, the 5 D’s of Prevention need to be considered (19). Further analysis of gender also indicated the numbers of males were notably higher then females across each age grouping. The demographic analysis for age shows the number
of human WNND cases increased with age by group (> 55 years old was the highest age group). WNF, however, was more frequent among individuals 31 to 49 years old. This corresponds with the Center for Disease Control and Prevention’s assessment of age as a risk factor (CDC, 2001a).

Examination of the county incidence rates for laboratory confirmed cases of WNND and WNF shows an increase in reported cases each year. The annual incidence rates show a linear increase over time. Florida will quickly develop a high disease burden if this incidence trend continues over the next few years. Basic counts by county show the same trend, but do not take into consideration that reporting of mild disease (WNF) may underestimate the problem for the more rural areas of Florida. The state has large geographical regions with low population densities due to large agricultural land uses. Levels of incidence provide less biased data than numbers of cases per county because they are based on population. However, county medical alerts are based in part on the number of diagnosed cases per county. The number of clinical cases is also important to consider for control and prevention.

**Temporal Distribution**

The weekly temporal distribution of confirmed West Nile events was similar across each surveillance type (sentinel chicken, dead avian and mosquito) with almost no events occurring from January to May. Analysis showed a transmission season beginning at approximately week 20 (late May to early June) and ending at week 52 (late December). The peak detection periods varied for each surveillance type, but when the focus was narrowed to look at only the transmission season, each system had a distinct peak period. A few outliers were observed outside the transmission season. These were
not included as part of the temporal distribution analysis because they occurred far outside the observed curve of the peak transmission period. These events may be random, carried over from the previous year’s transmission season or part of normal background activity and thus do not play a role in determining the temporal transmission to humans. Since West Nile virus is new to Florida, historical data is not yet available to determine normal thresholds of virus activity. Data should therefore continue to be collected in order to properly determine the baseline for WNV activity in Florida.

The distribution of confirmed WNND and WNF cases by week of onset did not appear to have a distinct peak period however, all of the reported cases had the same temporal distribution as other positive surveillance events. The lack of a clear peak range with in the transmission season may be a direct reflection on the low numbers of cases observed over the three year period. It is interesting to note that the number of reported cases has tripled with each successive year. This implies this trend will remain unknown without further data and investigation. This implicates that Florida will likely see an increase in the overall burden of disease in the future. Continuation of surveillance programs would allow for better case finding and more testing availability leading to more conclusive results and an improved ability to predict the risk of disease. The number of West Nile cases in Florida for 2004 has been lower then the previous years. Figure 43 shows the epi curve clinical cases of West Nile Disease where the number of cases have decreased during 2004 as compared to 2003. This may be due to herd immunity to West Nile or better control measures (e.g. mosquito control).
Figure 46: Epi Curve Comparing 2003 and 2004 Confirmed Human Cases.


**Peak Transmission**

A mean of the weeks with the highest number of cases of confirmed WNND and WNF for all three years (2001-2003) was the 37th week (mid-September). By combining the weeks with the greatest number of clinical cases from each year, human cases would be expected between the 28th and 44th week.

The mean transmission peaks varied for each surveillance type with sentinel chicken surveillance showing a mean peak in the 40th week (early November), dead bird surveillance showing a mean peak in the 36th week (early September), and mosquito surveillance showing a mean peak in week 33 (mid July). Collectively the surveillance
types have a mean transmission peak in week 36 with a range from week 29-46. The transmission range provided an indication that prevention and control efforts for West Nile and other arboviruses during and leading up to this time period would be most effective.

Dead bird and mosquito surveillance mean peaks were one and five weeks, respectively, prior to the mean peak in reported human cases. Conversely, the sentinel mean peak was three weeks after the mean peak in reported human cases. There was a substantial increase in the number of sentinel chicken sites in 2002 and 2003 which may indicate the data from the years considered (2001, 2002 and 2003) are not comparable. The peak of sentinel seroconversions is not as good an indication of risk as when positive outcomes first begin to appear and how quickly the outcomes increase. Ascertaining when separate surveillance systems peak in relation to when confirmed WNND and WNF reports peak is an indication of which surveillance system’s data may be the most useful in a model for the early detection of West Nile associated illnesses in Florida.

Unfortunately, more data will be needed to verify that dead bird and mosquito surveillance consistently peak prior to human cases, since participation in these surveillance activities also differed greatly between counties and years. Over one half of the human cases from 2001 and 2002 occurred in areas with no sentinel surveillance. In order to better utilize the sentinel surveillance program a baseline assessment over the next few years would help show increases above the normal background activity. Since WNV is new in Florida, the activity we have seen may not be normal therefore, the next years are imperative in establishing this threshold.
Since 2001 mosquito pool submissions and sentinel sites have increased in number by over 40% each year. This has provided better coverage in the counties participating in these surveillance types. The dead bird submissions have drastically decreased by almost 40% per year. The first year of WN detection (2001) had most of the dead bird submissions after the first dead crow was found positive, there were over 7,000 birds submitted in a six month period. Comparing the percent positive and number positive for dead birds showed a positive correlation with number of submitted dead birds and the percent positive. During 2001 there was greater number of positives then in the subsequent years (2002 & 2003) but the percent positive was lower. This means the excessive numbers of dead birds that were submitted may have diluted the results. The percent positive in 2003 was higher then the other two years, this may be due to the lower numbers or the species of dead birds submitted for testing. Over the last few years trends with particular bird species more prone to WN infection (e.g. corvids) have become well known resulting in some counties submitting only these species. Evaluating the percent WN positive and the total submitted dead birds for the three years observed suggests that 2003 was a comparable model, having the highest percent among the years even though the least number were submitted. This indicates the methods used during 2003 may be the best in order to show more specific results and keep the associated costs at a minimum.

Mosquito surveillance, WN percent positive was more consistent over the three years with 2001 and 2003 having the highest rates. However, there was no significant correlation for 2001 probably due to the lower numbers of submitted mosquito pools. The highest rate for mosquito positives was 0.07 showing a smaller range then the dead birds
where the highest rate excluding the first positive specimen (1.0), was 0.60. These results suggest that an increase in mosquito pool submissions would show better detection rates without diluting the outcome.

Mosquito control responses to sentinel seroconversions could possibly lead to a reduction or elimination of the number of human cases of WN disease. Consequently, these proactive efforts could influence surveillance data by stimulating an increase in sampling and submissions which might reduce the percentage of positive mosquito pools and dead birds.

**Regional Analysis**

Based on the physiographic climate, Florida’s 67 counties were separated into four regions: panhandle, north, central and south (Day, 1996). The cartographic representation is shown in Appendix IX. Each region’s surveillance data for dead avian, mosquito and sentinel chickens was examined independently to control for the bias of differing participation levels. The data sets were evaluated based on their predictive characteristics for human West Nile disease among regions. A list of the counties grouped by region with surveillance participation levels is located in Appendix VIII.

In general, interpretation of the regional surveillance data indicated that dead avian and sentinel chicken surveillance have the best predictive qualities. The graphs which show an increase in the number of sentinel seroconversions and positive dead birds before human cases were detected for each region (Fig. 29a,b; 30b,c; 31b,c; 32b,c). There were incidents where the initial clinical case was seen before an increase in surveillance activity. This may be due to clinical cases observed early in the season or lack of participation from the region at the time. The latter may be an anomaly associated with
the submission of dead birds because of media attention given to West Nile whenever a new clinical case is diagnosed.

Surveillance data collected in 2001 from all three systems proved to be too unreliable to evaluate, limitations from this year may be due to increased sample submissions after reported cases of WNND and WNF. Some regions showed human case foci initially in areas without surveillance programs.

Mosquito surveillance data proved unreliable for use as a predictor for human cases because of low detection numbers of WNV as well. This may be based on several factors including the number of mosquitoes needed for testing, location, collection methods, and storage conditions. All of these issues can individually or collectively have an adverse affect on the samples leading to the inability to detect the virus.

Florida’s southern region appears to have the worst predictive qualities of the four regions. The explanation may lie with the fact that the southern region continually began surveillance sample submission later than the other regions. Additionally the counties within the region were inconsistent with the level of participation for each surveillance type. Excluding mosquito data, the best region for predicting clinical cases from surveillance data in Florida was the panhandle. This is also seen in Appendix VIII which shows the levels of participation by counties within each region.

The regional graphs showed several peaks for sentinel positives within the distribution for some of the years examined. These may be an anomaly caused by the replacement of birds with in a flock after the positive birds are removed. The first week the chicken is bled is used as the baseline sera. The birds are then placed with exposed flock for weekly serosurveillance. The new sentinel bird may seroconvert after this time.
Thus, there is a 2 week lag between removing seroconverted sentinels and adding new ones which may account the decrease in positives during this time period.

Since sentinel surveillance is controlled by the submitting counties it is important to consider the point during the year at which counties begin their surveillance. Table 1 lists all the counties participating in sentinel surveillance and the month they start submitting sentinel sera. Counties that participated during 2001, 2002 and 2003 began the surveillance before clinical cases were seen with the exception of DeSoto, Santa Rosa and Washington counties during 2002. These counties were not consistent in submitting their sera in a timely manner allowing breaks in weeks before submitting the next specimen.
Table 1. Month of First Sera Submitted for Counties Participating in Sentinel Surveillance for 2001, 2002 and 2003 Organized by Region.

<table>
<thead>
<tr>
<th>County</th>
<th>2001</th>
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<th>County</th>
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Limitations with this type of analysis include a lack of a representative sample for mosquitoes and dead birds. Raw numbers were used instead of rates in order to show all of the surveillance types in one graph. There was no way to correctly estimate the numbers needed for the denominator for each region, county or statewide by week. This is important to consider because sample submissions by county range depending on several factors. Urban areas generally submit more dead birds, increased media attention also stimulates submissions; but rural areas may not have the available funds to support
these increases. Therefore, the surveillance data may over or underestimate the actual disease burden within individual regions.

**Spatial Analysis**

The best overall indicator from the spatial analysis of clinical cases was positive dead birds which had a positive correlation showing an increase in both diagnosed cases of West Nile infection and positive dead birds. This is especially noticeable in Escambia and Bay Counties which had the highest numbers of human WN cases during 2002 and 2003. Some counties had lower submissions of avian which may account for the lack of correlation with clinical cases. There may also be an association between the number of dead bird submissions and urban population centers. It is more probable for dead birds to be found and submitted in urban areas than in rural areas.

Sentinel serosurveillance also had some value; unfortunately, the counties with the most human cases did not participate in the program. There was a negative correlation for the cumulative years, 2001 and 2002. The correlation for 2003 was positive with all the data considered but negative when outliers were excluded. A better correlated model may be ascertained if all the counties in the state participated. Another explanation for the negative correlation may be associated with increased control measures after sentinels seroconvert therefore, preventing cases. This would show an increase in sentinel seroconversion and a decrease in clinical cases.

The mosquito pool surveillance data set was not a useful indicator for clinical cases for any of the years observed. There were not enough positive mosquito pools to accurately show correlations between the clinical cases and positive mosquitoes. There may be a need for better collecting methods or increasing the number of mosquitoes.
submitted to facilitate a improved detection of West Nile virus. Another explanation for
the negative mosquito correlations, as shown in the sentinel population, could be
associated with increased control measures after a positive mosquito pool is detected.

Spatial analysis does not allow for a sequential time period to be evaluated
therefore, the temporality for casual inference may introduce bias into the models. This is
especially important to consider when using the surveillance systems as a predictor
because detection of WN virus activity may not necessarily come before the clinical
cases. This type of analysis is useful for obtaining an historical overview, comparing
geographic locations and establishing overall participation in surveillance by county or
region.

The correlations showed outlier observations for all of the years and the
cumulative years for positive dead bird and mosquito pool surveillance. The sentinel
serconversion and clinical case correlations showed outliers for the cumulative and 2003
analysis. Excluding these observations resulted in better fitting regression lines.
However, the overall correlation was similar to the original with exception of sentinel
serconversion rates and clinical cases during 2003 which changed from no correlation to
a negative correlation. This effect may be important to consider because the outlying
observation could skew the overall correlation.

Poisson Distribution Regression Model

Due to the response variable (incidence of human cases) being a rate and a rare
event, the regression model was done using the Poisson distribution in order to accurately
show the predictive values for the surveillance systems. The data was organized by
region using cumulative (2001, 2002 and 2003) and weekly observations. Regression
analysis showed that each surveillance type showed different value as a predictive model for human disease. The actual outcome from the regression analysis was similar to the expected for positive dead birds and sentinels which were shown to be good predictors for clinical cases. Also expected was the outcome for Mosquito surveillance which was not an overall good predictor of human disease.

The analysis for 2001 and 2002 did not show any of the surveillance systems as predictors (p-values >0.10), except for 2001 with region included, this may due to the lack of human cases which resulted in very small rates for incidence. This phenomenon may also be a consequence of increased submissions of specimen after the first clinical case because of the media attention especially in the first year (2001) of WN detection.

The adjusted model including the data from all surveillance types showed that positive dead bird surveillance was the best predictor for human cases in 2003. The cumulative year adjusted model was similar to the univariate analysis. These results from the 2003 data indicate that positive dead birds were the best predictor for clinical cases.

The effect of region in the model was seen in cumulative analysis for mosquito and sentinel surveillance, and the dead bird surveillance in 2003. However, mosquitoes were improved as a predictor for the individual year analysis for human cases with region considered was mosquito pools. The data also showed in 2001 sentinels were better predictors when region was adjusted for the region having the most effect comparatively was the panhandle. These results may be associated with confounding because of a greater number of clinical cases or number of positives (mosquitoes-2003 and sentinels-2001) in the panhandle. This would be adjusted for by including region.
Study Strengths and Limitations

This study’s major strength was indicating where improvements to the overall West Nile surveillance system could be implemented to enhance public health. It also indicated that the current surveillance system has been successful in predicting human disease in the areas that consistently participate. The use of separate analyses for each surveillance type showed that the dead bird, sentinel and mosquito pool surveillance projects were multifaceted. This means that while one system may not indicate a correlation, others may. It also provides a basis for future studies on the natural history of West Nile Virus.

There were several limitations encountered in this study. The data proved unreliable in showing a representative rate because there was no available population information for mosquitoes and birds. The rates used for positive birds may have over or under estimated West Nile activity depending on the county, because submissions may have been larger in the urban areas and smaller in rural areas of Florida.

Mosquito pool surveillance data may have been biased because the submissions were sporadic and were mostly based on the individual county interests; therefore the mosquito pools submitted may not have been for surveillance purposes only. Mosquito specimens have many problems as a surveillance program including the need for large submission number in order to detect West Nile and specific sample handling and shipping procedures which are not mandated in any form.

Human surveillance for 2001 and 2002 had low incidence rates and were not reliable for predictive models. Another problem was due to the varied participation levels
within the Florida regions and counties. This included counties with human cases that did not use any of the surveillance systems.

Specific problems were observed with in the sentinel surveillance program. The overall data were reliable however; Santa Rosa and Washington counties were not consistent in their participation. Santa Rosa county submitted sera from six chickens at three separate times during 2002 and four separate times during 2003 each was about three to four weeks apart. Washington County did the same for 2003. This does not constitute participation in the sentinel surveillance program.

**Evaluation**

An overall evaluation of the performance of the Arbovirus surveillance system for WNV shows that each surveillance type (dead bird, mosquito and sentinel chickens) provides important information about virus transmission in the environment. Positive mosquito trends tend to provide an earlier warning system when adequate sampling is performed. Positive dead birds appear to be better predictors for human cases and sentinel chicken serosurveillance indicates activity within a specific geographic area. These systems are costly to maintain however, continuation will help to lower the disease burden and risk of disease resulting in significantly decreased health care expenditures. The programs initiated for education by the Florida Department of Health and the Center for Disease Control and Prevention are also useful tools to keep up public awareness and decrease the number of possible cases. The surveillance program has proven to be stable and successful since Florida has been using these surveillance techniques for other endemic arboviruses for over 30 years.
Nevertheless, this evaluation illuminates several areas in which enhancements can be made to help show even better correlations and predictive values for the overall system.

- Increase the coverage and consistency of submissions for all surveillance types.

- Set standard levels of participation for all counties based on the regional analyses and populations at risk.

- Create standardized approaches for sampling, shipping and submitting samples (especially for mosquito pool submissions) and require that participating counties adhere to these standards.

- Only submit specific birds known to be especially susceptible to West Nile Virus (e.g. corvids).

- Targeted prevention and education strategies for higher risk groups based on their potential levels of exposure.
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Appendices
Appendix I: Table of the Species of Birds that were found Positive for West Nile Virus in the United States since 1999

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<th>Bird Species Common Name</th>
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### Appendix I: (Continued)

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<td>Red-eyed Vireo</td>
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<td>Red-headed Woodpecker</td>
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<td>Smew</td>
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### Appendix I: (Continued)

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Appendix I: (Continued)

<table>
<thead>
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<th>Bird Species Common Name</th>
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<td>276 Yellow Warbler</td>
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<td>277 *Yellow-bellied Sapsucker</td>
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<td>278 *Yellow-billed Cuckoo</td>
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<td>279 *Yellow-billed Duck</td>
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<td>281 Yellow-crowned Night-Heron</td>
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<tr>
<td>282 *Yellow-rumped Warbler</td>
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<td>283 *Zebra Finch</td>
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<td>284 Zenaida Dove</td>
<td>Exotic-captive</td>
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*Found positive in Florida

Adapted from the CDC compiled list of West Nile Virus Positive Bird Species. Available from URL: http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm
Appendix II: Table of the Species of Mosquitoes that were found Positive for West Nile Virus in Mosquito Pools in the United States since 1999

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Aedes albopictus</th>
<th>Aedes aegypti</th>
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<td><em>Anopheles barbieri</em></td>
<td><em>Anopheles atropos</em></td>
<td><em>Anopheles crucians/bradleyi</em></td>
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<tr>
<td><em>Anopheles punctipennis</em></td>
<td><em>Anopheles quadrimaculatus</em></td>
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<tr>
<td><em>Anopheles walkeri</em></td>
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<td>Coquillettidia perturbans</td>
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<td><em>Culiseta inornata</em></td>
<td><em>Culiseta melanura</em></td>
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<td><em>Culex</em></td>
<td><em>Culex erraticus</em></td>
<td><em>Culex nigripalpus</em></td>
<td>Culex pipiens</td>
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<td><em>Culex quinquefasciatus</em></td>
<td>Culex restuans</td>
<td><em>Culex salinarius</em></td>
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<td><em>Culex tarsalis</em></td>
<td>Culex territans</td>
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<td><em>Deinocerites</em></td>
<td><em>Deinocerites cancer</em></td>
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<td><em>Ochlerotatus atlanticus/tormentor</em></td>
<td>Ochlerotatus canadensis</td>
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<td><em>Ochlerotatus cantator</em></td>
<td>Ochlerotatus dorsalis</td>
<td>Ochlerotatus fitchii</td>
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<td><em>Ochlerotatus infirmatus</em></td>
<td>Ochlerotatus japonicus</td>
<td>Ochlerotatus provocans</td>
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<td><em>Ochlerotatus sollicitans</em></td>
<td>Ochlerotatus sticticus</td>
<td>Ochlerotatus stimulans</td>
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<td><em>Ochlerotatus taeniorhynchus</em></td>
<td>Ochlerotatus triseriatus</td>
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<td><em>Psorophora columbae</em></td>
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<td><em>Psorophora howardii</em></td>
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<td><em>Uranotaenia</em></td>
<td>Uranotaenia sapphirina</td>
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*Found positive in Florida

Table adapted from CDC List of Species of West Nile Positive Mosquito Pools. Available from URL http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm
Appendix III: West Nile virus reservoir competence index values for 25 species of birds

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Mean Days Infectious*</th>
<th>Mean Peak Viremia **</th>
<th>ci***</th>
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<td>2.4</td>
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<td>6</td>
<td>8.8</td>
<td>0.8</td>
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<td>6.7</td>
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<td>2.7</td>
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*Infectious viremia = log 5 or greater per ml serum; ** log pfu/ml serum
*** ci = susceptibility * mean infectiousness * days infectious

*Table adapted from (Komar, 2003).
## Appendix VI: 2001 data by County

<table>
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<tr>
<th>County</th>
<th>Region</th>
<th>Clinical Cases</th>
<th>Avian Positive</th>
<th>Mosquito Positive</th>
<th>Sentinel Rate</th>
<th>Sentinel Positive</th>
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● participation in the surveillance system
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Appendix XI: Graphs of Pooled Annual Averages (2001-2003) with Moving Averages for West Nile Surveillance Data Statewide and Regional per Week


![Plot of the Combined Year (2001-2003) Average and the Moving Average Models for the Number of Clinical Cases in Florida.](image)

Plot of the Combined Year (2001) Average and the Moving Average Models for the Number of Positive Dead Birds in Florida.

![Plot of the Combined Year (2001) Average and the Moving Average Models for the Number of Positive Dead Birds in Florida.](image)
Appendix XI: (Continued)


Appendix XI: (Continued)


Plot of the Combined Year (2001) Average and the Moving Average Models for the Number of Positive Dead Birds in the Panhandle Region of Florida.
Appendix XI: (Continued)


![Graph showing sentinel seroconversion rate with actual and moving average lines.]


![Graph showing number of positive mosquito pools with actual and moving average lines.]

158
Appendix XI: (Continued)

Plot of the Combined Year (2001-2003) Average and the Moving Average Models for the Number of Clinical Cases in the Northern Region of Florida.

Plot of the Combined Year (2001) Average and the Moving Average Models for the Number of Positive Dead Birds in the Northern Region of Florida.
Appendix XI: (Continued)

Plot of the Combined Year (2002) Average and the Moving Average Models for the Sentinel Seroconversion Rate in the Northern Region of Florida.

Plot of the Combined Year (2003) Average and the Moving Average Models for the Number of Positive Mosquito Pools in the Northern Region of Florida.
Appendix XI: (Continued)

Plot of the Combined Year (2001-2003) Average and the Moving Average Models for the Number of Clinical Cases in the Central Region of Florida.

Plot of the Combined Year (2001) Average and the Moving Average Models for the Number of Positive Dead Birds in the Central Region of Florida.
Appendix XI: (Continued)


![Plot of the Combined Year (2002) Average and the Moving Average Models for the Sentinel Seroconversion Rate in the Central Region of Florida.]

Plot of the Combined Year (2003) Average and the Moving Average Models for the Number of Positive Mosquito Pools in the Central Region of Florida.

![Plot of the Combined Year (2003) Average and the Moving Average Models for the Number of Positive Mosquito Pools in the Central Region of Florida.]
Appendix XI: (Continued)


Plot of the Combined Year (2001) Average and the Moving Average Models for the Number of Positive Dead Birds in the Southern Region of Florida.
Appendix XI: (Continued)


### Poisson Regression Output for Pooled Years (2001-2003)

**Human Incidence, Avian, Mosquito with Sentinel Rates including Region**

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**Human Incidence with Avian Rates including Region**

| Intercept            | 1  | -61.0452 | 2.1902 | -65.3380     | -56.7524     | 776.82| < 0.0001   |
| Region: Central      | 1  | -8.9956  | 0.3446 | -9.6710      | -8.3201      | 681.38| < 0.0001   |
| Region: North        | 1  | -7.0472  | 0.3177 | -7.6699      | -6.4244      | 491.95| < 0.0001   |
| Region: Panhandle    | 1  | -6.5669  | 0.3933 | -7.3377      | -5.7960      | 278.82| < 0.0001   |
| Region: South        | 0  | 0.0000   | 0.0000 | 0.0000       | 0.0000       |       |            |
| Year                 | 1  | 26.8621  | 0.7923 | 25.3092      | 28.4150      | 1149.48| < 0.0001   |
| Avian Rate           | 1  | -0.0496  | 0.0034 | -0.0564      | -0.0429      | 207.97| < 0.0001   |
| Scale                | 0  | 1        | 0      | 1            | 1            |       |            |

**Human Incidence with Mosquito Rates including Region**

| Intercept            | 1  | -48.5445 | 3.9168 | -56.2214     | -40.8677     | 153.61| < 0.0001   |
| Region: Central      | 1  | -2.2198  | 0.2308 | -2.6722      | -1.7675      | 92.53 | < 0.0001   |
| Region: North        | 1  | -1.0844  | 0.1567 | -1.3914      | -0.7773      | 47.92 | < 0.0001   |
| Region: Panhandle    | 1  | -1.8177  | 0.1127 | -2.0385      | -1.5969      | 260.38| < 0.0001   |
| Region: South        | 0  | 0.0000   | 0.0000 | 0.0000       | 0.0000       |       |            |
| Year                 | 1  | 19.8966  | 1.3111 | 17.3270      | 22.4663      | 230.31| < 0.0001   |
| Mosquito Rate        | 1  | 0.0870   | 0.0048 | 0.0775       | 0.0965       | 322.38| < 0.0001   |
| Scale                | 0  | 1        | 0      | 1            | 1            |       |            |

**Human Incidence with Sentinel Rates including Region**

| Intercept            | 1  | -52.8139 | 2.0459 | -56.8239     | -48.8039     | 666.36| < 0.0001   |
| Region: Central      | 1  | 0.3472   | 0.1920 | -0.0290      | 0.7236       | 3.27  | 0.0706     |
| Region: North        | 1  | -0.9973  | 0.1293 | -1.2507      | -0.7438      | 59.50 | < 0.0001   |
| Region: Panhandle    | 1  | -1.4530  | 0.1093 | -1.6673      | -1.2388      | 176.74| < 0.0001   |
| Region: South        | 0  | 0.0000   | 0.0000 | 0.0000       | 0.0000       |       |            |
| Year                 | 1  | 22.3450  | 0.6838 | 21.0048      | 23.6853      | 1067.78| < 0.0001   |
| Sentinel Rate        | 1  | -0.0223  | 0.0010 | -0.0242      | -0.0204      | 535.95| < 0.0001   |
| Scale                | 0  | 1        | 0      | 1            | 1            |       |            |

**Human Incidence, Avian, Mosquito with Sentinel Rates**

| Intercept            | 1  | -51.8830 | 3.9198 | -59.5656     | -44.2003     | 175.20| < 0.0001   |
| Year                 | 1  | 21.4351  | 1.3125 | 18.8627      | 24.0075      | 266.73| < 0.0001   |
| Avian Rate           | 1  | 0.0099   | 0.0008 | 0.0083       | 0.0116       | 139.60| < 0.0001   |
| Mosquito Rate        | 1  | 0.0120   | 0.0044 | 0.0035       | 0.0206       | 7.60  | 0.0058     |
| Sentinel Rate        | 1  | -0.0184  | 0.0011 | -0.0206      | -0.0162      | 264.47| < 0.0001   |
| Scale                | 0  | 1        | 0      | 1            | 1            |       |            |
Appendix XII: (Continued)

### Poisson Regression Output for Pooled Years (2001-2003) continued

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<th>Upper WaldCL</th>
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<th>Prob ChiSq</th>
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### Poisson Regression Output for 2001

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Appendix XII: (Continued)

### Poisson Regression Output for 2001 (continued)

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## Poisson Regression Output for 2003

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### Human Incidence and Avian Rates with Region

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