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Ecology and Epidemiology of West Nile Virus in Mississippi

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Ecology and epidemiology of West Nile virus in Mississippi

By

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Since its introduction in 2002, West Nile virus (WNV) persists in Mississippi with dozens of cases and a few deaths each year. Little is known about the epidemiology and ecology of WNV in our state. This is the first study of the dynamics of West Nile virus in Mississippi utilizing both mosquito and human case surveillance. Herein I showed that the primary vector for WNV in MS, *Culex pipiens quinquefasciatus*, is associated with anthropogenic urban environments as opposed to rural wooded areas. I also found that other potential WNV vectors in MS are likely involved in enzootic transmission among natural bird reservoirs and not related to human transmission.

Secondly, I showed that a simple commercial wicking assay (dip-stick test) can be beneficial to vector surveillance and mosquito control programs with limited resources when monitoring local mosquito populations in anticipation of human disease transmission. In my study, the lead time from finding infected mosquitoes to onset of human cases ranged from almost two weeks to two months, an important finding in regard to public health.

Lastly, I performed a descriptive analysis on data from a survey I sent out to Mississippi WNV patients who were diagnosed between 2008 and 2013. The survey

focused on self-reported personal protective behaviors and descriptions of their home and property at the time of infection. Results highlighted a few key epidemiological and behavioral aspects of WNV patients in Mississippi: 1) television and internet were the primary avenues for patient education; 2) amount of time spent outdoors appeared associated with WNV infection; and 3) use of personal protection measures did not usually change from before WNV infection to after. This study will help public health personnel achieve their goals to promote health and educate the public about personal protective behaviors for WNV and other mosquito-borne diseases, and thus, reduce risk of future infections. It will also lay groundwork for future studies such as widespread sero-surveys of populations to assess WNV infection rates and onsite environmental surveys to validate patient responses. Also, hypothesis-driven studies of specific risk factors associated with WNV infection are in order and currently planned.

DEDICATION

I dedicate this dissertation to my mother, Deborah, who always had faith in me, was my biggest cheerleader, and who loved me unconditionally. She was diagnosed with a rare brain tumor in May of 2009 and on May 14, 2014, after a long hard fight, the tumor finally won. I can only hope she left this world knowing how much she was loved and how much she will be missed.

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CHAPTER I

INTRODUCTION AND BACKGROUND

1.1 Introduction and dissertation overview

Since the introduction of West Nile virus (WNV) into the U.S. in 1999, there have been multiple nationwide studies focused on determining what causes localized epidemics or hotspots around the country (Dohm et al. 2002, Shaman et al. 2003, 2005, Reisen et al. 2006, Soverow et al. 2009); however, almost none of them have been done in the southeastern states (DeGroote et al 2014). Much remains unknown concerning risk factors such as demographic and local environmental characteristics and human WNV disease, especially in the southern U. S. Since its introduction into the state, certain areas of Mississippi have consistently been considered West Nile virus (WNV) hotspots, and thus, these areas are prime spots for WNV research.

This manuscript includes the following chapters: 1) a review of WNV with particular emphasis on Mississippi; 2) results of a study to determine abundance and seasonality of potential WNV vectors in non-urban wooded areas; 3) results of testing mosquitoes using a commercial wicking assay for advanced warning of human WNV infection; and 4) results of a descriptive analysis of surveys of properties and behaviors of human WNV cases occurring in Mississippi 2008-2013.

1.2 Mosquito-borne diseases in general

Mosquitoes are the number one arthropod vectors of human disease worldwide, with hundreds of millions of cases of disease and approximately a million deaths each year, mostly from malaria and dengue (Service 2001, Foster and Walker 2002, Kampen and Schaffner 2008, Guerrant et al 2011). In North America, problems from mosquito-borne illnesses historically originated from malaria and yellow fever, although these illnesses have mostly been eliminated here (Guerrant et al 2011, Goddard 1998). In addition, there are numerous mosquito-borne arboviruses in North America such as Eastern equine encephalitis virus, St. Louis encephalitis virus, and West Nile virus (WNV). The term arbovirus is derived from the phrase, arthropod-borne virus. These viruses are maintained in natural cycles involving mosquito vectors and susceptible vertebrate hosts. A vertebrate host acquires the virus while being fed upon by an infected mosquito (Calisher 1994). Spillover from natural vertebrate-host cycles can cause accidental human and other vertebrate infections and generally occurs when there is a rapid, progressive, and accumulative increase in the number of infected hosts and vectors (Tsai and Mitchell 1989). There have been approximately 60 arboviruses isolated within the United States, however only those belonging to families Flaviviridae (flaviviruses), Togaviridae (togaviruses), and Bunyaviridae (bunyaviruses, including the California serogroup viruses) cause significant neurological or encephalopathic disease in humans (Calisher 1994, Gubler et al. 2007).

1.3 West Nile virus

West Nile virus (WNV) is a mosquito-borne, enveloped single-stranded, positive-sense RNA virus belonging to the Flaviviridae family of viruses. The Flaviviridae consists of three genera: *Flavivirus* (including WNV), *Hepacivirus* and *Pestivirus*. Genus *Flavivirus* is further divided into 12 serocomplexes including the Japanese Encephalitis virus (JEV) serocomplex which also includes Kunjin virus, Murray Valley Encephalitis, St. Louis Encephalitis (SLEV), Cacipacore, Koutango, Usutu and Yaounde viruses. Kunjin virus is actually a subtype of WNV and is endemic to Australia (Scherret et al. 2001, Fauquet 2005).

1.3.1 West Nile virus lineages and strains

Studies have suggested that WNV may have emerged as a distinct virus around 1000 years ago (Marr and Calisher 2003). Traditionally, phylogenetic studies showed two main lineages of WNV consisting of several strains (Berthet et al. 1997, Hayes et al. 2005b), although some researchers now say there might be as many as five or more lineages (Mackenzie and Williams 2009, May et al. 2011). Strains from Lineage I can be found in Africa, India, Australia, and the Western Hemisphere and are responsible for the outbreaks in Europe and in the Mediterranean basin (Komar 2000). Lineage I is made up of a group of diverse sublineages demonstrating extensive heterogeneity in antigenic character and nucleotide sequences (Lanciotti et al. 2002). This can be observed in the Indian and Australian (Kunjin subtype) versus other sublineages. Sublineages in Lineage I have been further divided into three clades (Lanciotti et al. 2002). Clade 1a consists of isolates from Africa, Europe, Asia and the Americas, clade 1b contains isolates of Kunjin viruses and clade 1c consists of isolates from India.

Strains from Lineage II were originally only reported in Madagascar, sub-Saharan Africa and Uganda where the first WNV isolation was made in 1937 (Lanciotti et al. 1999, Brinton 2002, Murgue et al. 2002, Komar 2003), however, more recently Lineage II isolates have also been identified in Europe. Historically, Lineage II isolates were generally associated with asymptomatic infections but the European isolates have been shown to be more virulent (Bakonyi et al. 2005, Erdélyi et al. 2007). In 1998, a virulent WNV strain from Lineage I was identified in migrating storks and domestic geese showing clinical encephalopathic symptoms and paralysis in Israel. A nearly identical WNV strain emerged in New York in 1999, causing the deaths of thousands of native birds and multiple cases in humans with some deaths (see discussion in following sections). Figure 1.1 illustrates the relationship between the 1998 strain from Israel and the strain isolated from New York in 1999 (Lanciotti et al. 1999). The virus has spread rapidly and is now well established in the Western Hemisphere (Zeller and Schuffenecker 2004). Several distinct strains of Lineage I WNV have been isolated across the U.S. since its initial discovery in 1999 including MS (Añez et al. 2013, Mann et al. 2013).

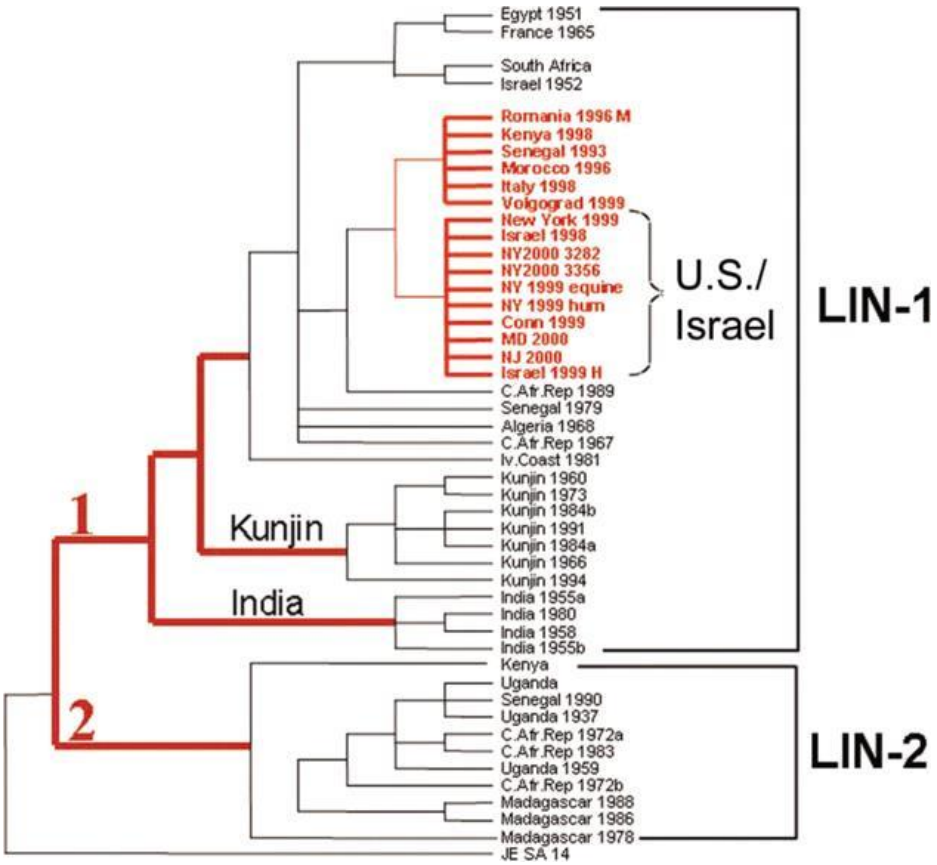

Figure 1.1 Phylogenetic tree of West Nile virus.

Source: Centers for Disease Control and Prevention.

There is strong evidence to suggest that a dominant WNV strain has developed throughout most of North America as a result of several nucleotide mutations within the genome (Davis et al. 2005). This has led to a displacement of the original and most subsequent strains due to a competitive advantage exhibited by the newer dominant strain. Some studies have shown that the extrinsic incubation period of the dominant strain is shorter than that of most others leading to increased transmission efficiency. One study in Houston, TX has demonstrated the possibility of a genetic stasis within the virus due to the lack of newly formed subclades from isolates of previous years. It is

unclear if this is the case for all of North America or limited to a certain geographic region (Davis et al. 2007).

1.3.2 History and Background of WNV

WNV was first isolated from a febrile woman from the West Nile province of Uganda in 1937 (Smithburn et al. 1940). The first epidemic of WNV occurred in Israel in the 1950's (Bernkopf et al. 1953, Zeller and Schuffenecker 2004). Outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994, Romania in 1996-1997, the Czech Republic in 1997, the Democratic Republic of the Congo in 1998, Russia in 1999, the United States in 1999-2003, and Israel in 2000 (Figure 1.2). Epizootics of disease in horses have occurred in Morocco in 1996 and 2003, Italy in 1998 (Cantile et al. 2000), the United States in 1999-2001, and France in 2000 (Durand et al. 2002) and in birds in Israel in 1997-2001 and in the United States in 1999-2002 (Hubalek and Halouzka 1999, Murgue et al. 2002, Schuffenecker et al. 2005). WNV is now known to be enzootic in birds throughout most of Africa, southern Europe, India, the Middle East, western and southeast Asia, Australia (subtype Kunjin virus), and seasonally endemic in North America (Mackenzie et al. 2004, Hayes et al. 2005a, Heymann 2008). WNV was first detected in North America in 1999 and the virus subsequently spread westwards across the United States, southward into Central America and the Caribbean, and northward into Canada (Hayes et al. 2005a, Davis et al. 2006, Gubler 2007). The largest ever recorded outbreak of WNV occurred in South Africa in 1974 with nearly 10,000 human cases (McIntosh et al. 1976).

Figure 1.2 Epidemics caused by WNV, 1937-2006.

Red stars indicate epidemics that have occurred since 1994 that have been associated with severe and fatal neurologic disease in humans, birds, and/or equines (Gubler, 2007).

1.3.2.1 Western Hemisphere/United States

West Nile Virus was first discovered in the Western Hemisphere in 1999 in New York City, NY, U.S.A., where there were a total of 59 cases and seven deaths (Asnis et al. 2000, Mostashari et al. 2001). Although initially attributed to St. Louis encephalitis (SLE) virus based on positive serologic findings in cerebrospinal fluid (CSF) and serum samples using a virus-specific IgM-capture enzyme-linked immunosorbent assay (ELISA), the cause of the outbreak was later confirmed to be a West Nile-like virus based on identification of virus in human, avian, and mosquito samples (CDC 1999). By 2002 the virus had spread to 45 of the 48 contiguous states, two provinces in Canada, and six states in Mexico (Estrada-Franco et al. 2003). Since its introduction, WNV has been detected in 326 different species of birds and has caused death in several mammal species

including horses, squirrels, dogs, cats, sheep, domestic rabbits, eastern chipmunks, bats, striped skunks, raccoons, harbor seals and at least one captive killer whale. Up to 45% percent of infected horses showing clinical signs results in death (Epp 2007).

Approximately 80% of all WNV infections are asymptomatic. The remaining 20 percent generally present to clinics with a mild flu-like illness that is accompanied by a range of symptoms. Most common symptoms include fever, headache, myalgia, muscle weakness, gastrointestinal disruption, and in some cases a maculopapular rash may develop on the chest, back, and arms. In the U.S., WNV has become the leading cause of epidemic meningoencephalitis in humans, however, it is estimated that less than 1% of all WNV infected patients develop the neuroinvasive form of the disease. The irreversible neurological damage resulting from meningoencephalitis, meningitis, or acute flaccid paralysis (AFP) is often severe (DeBiasi and Tyler 2006). Research suggests that AFP and muscle weakness associated with WNV involve damage to anterior horn cells of the spinal cord which often presents as a polio-like syndrome (Fratkin et al. 2004). It is estimated that 1 out of every 150 cases of WNV will develop into the more severe neuroinvasive form (Petersen and Roehrig 2001). There are no known specific treatments for WNV and the patient is generally treated only with supportive care.

1.3.2.2 History of WNV in Mississippi

WNV was first documented in Mississippi in humans in July of 2002 (CDC 2002a). The first 2 confirmed cases occurred in 2 men, 56 and 57 years old, from Hinds County, MS (CDC 2002b). By mid-August a total of 48 confirmed and probable cases had been diagnosed in Mississippi. The median age range was 55 years with the youngest being 3 and the oldest 89 (CDC 2002a). By the end of 2002, Mississippi had a

total of 192 WNV cases with 162 of those resulting in the neuroinvasive form of encephalitis; there were 12 deaths (CDC 2002a). In the decade since its introduction into Mississippi in 2002, WNV has continued to persist statewide. Clinical disease is also well established and considered endemic to our area. There has not been a leveling off of WNV cases, but rather a very consistent cyclic pattern as evidenced by up years and down years. The year 2012 was the tenth anniversary of the introduction of WNV in Mississippi and proved to be the worse year for human infections with a total of 251 cases and 5 deaths. Out of those 251 cases, 103 presented with the more severe form of neuroinvasive disease characterized by encephalitis (MSDH 2012). The graph below illustrates cyclic patterns of yearly WNV cases in Mississippi (Figure 1.3).

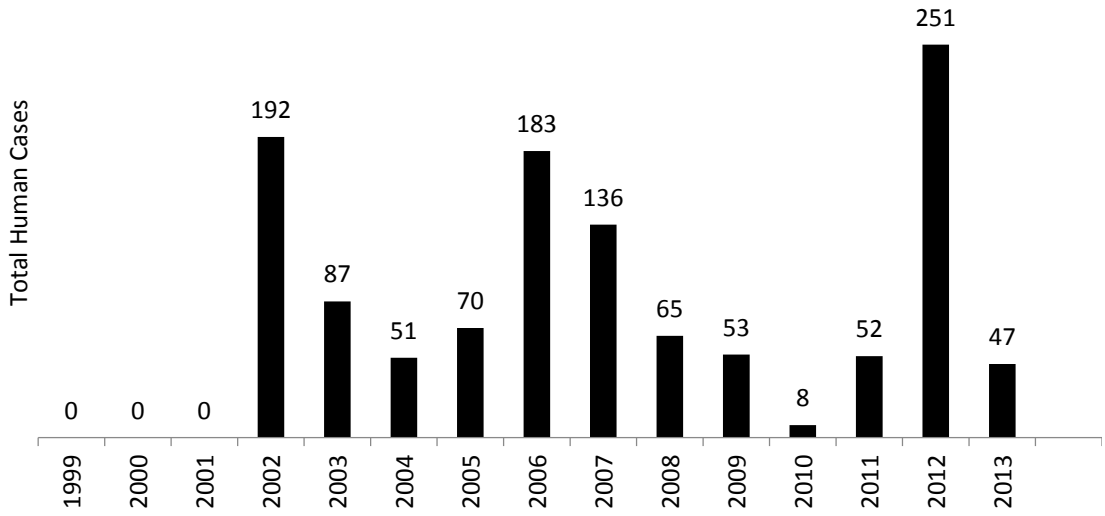

Figure 1.3 Total reported number of human WNV cases, Mississippi, 2002-2013.

The Lower Mississippi River Basin and some Midwestern states typically experience yearly high incidence rates of WNV compared to the rest of the country

(Sugumaran et al. 2009, Petersen et al. 2013). Figure 1.4 illustrates the average human incidence rate of WNV by county throughout the U.S. Parts of Mississippi and Louisiana distinctly stand out compared to the rest of the southeastern U.S., however it must be noted that this map does not include data for the less severe West Nile fever form of the disease.

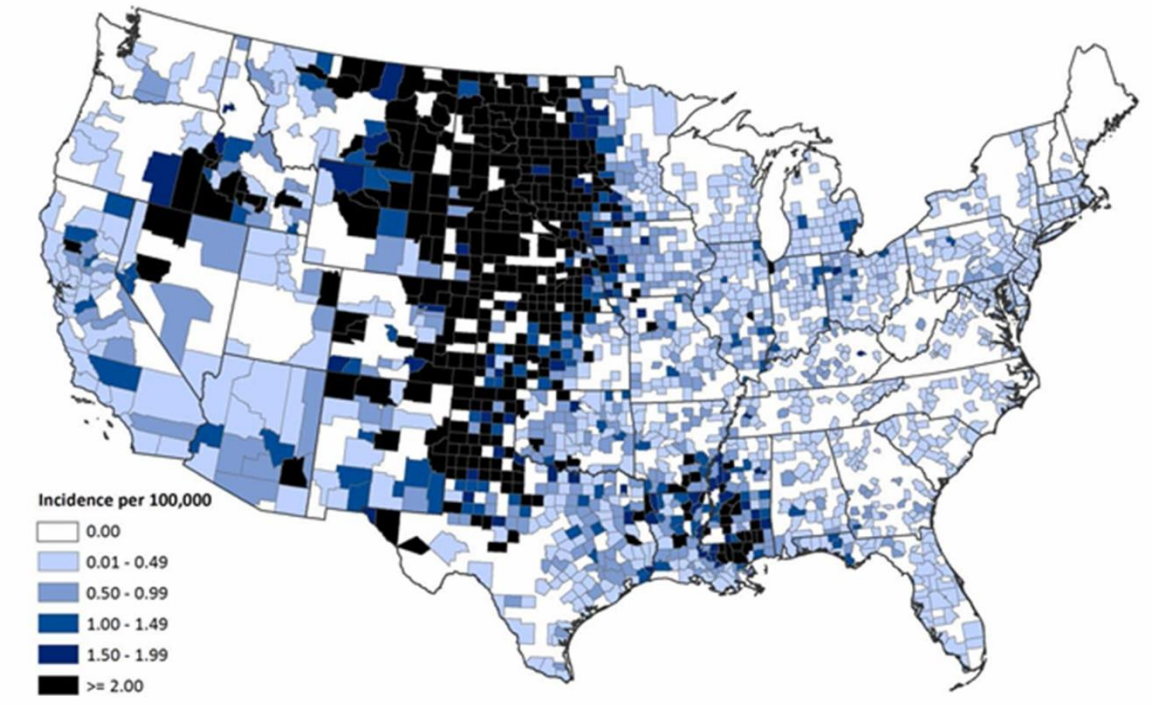

Figure 1.4 Average annual incidence of West Nile virus neuroinvasive disease reported to CDC by county, 1999-2013.

Source: Centers for Disease Control and Prevention.

1.4 Vectors of WNV

Mosquitoes are considered the most prolific arthropod vector for a number of flaviviruses (Hazell and Rogers 2005) in the U.S. As of 2009 WNV, 64 species of mosquitoes have tested positive for the virus in the U.S. (CDC 2014). Of those 64 species, 42 are known to occur in Mississippi (Goddard et al. 2009), but only a few are known competent vectors of WNV. Many factors affect vector competence in mosquitoes (Goddard 2002). As far as is known, ornithophilic *Culex* species are the primary enzootic vectors of WNV in the U.S. *Culex tarsalis* has been determined to be the primary vector of the virus in western and midwestern states (Goddard et al. 2002). The primary vectors in Mississippi are part of the *Culex pipiens* complex, which occurs in the Mississippi River Basin. The complex is made up of four primary taxa: *Cx. p. pipiens* form *pipiens* L., *Cx. p. quinquefasciatus* Say, hybrids between *Cx. p. pipiens* f. *pipiens*, and *Cx. p. quinquefasciatus*, and *Cx. p. pipiens* form *molestus* Forskål (Kothera et al. 2009, Mutebi and Savage 2009, Kothera et al. 2010).

Culex p. pipiens and Cx. p. quinquefasciatus were introduced into the U.S. presumably from Europe and Africa respectively (Ross 1964). In Mississippi, *Culex p. quinquefasciatus* is the primary vector for WNV (Rutledge et al. 2003, Godsey et al. 2005, Hayes et al. 2005a), and appears to be predominantly found in urban areas. Whether or not, and to what extent, this vector occurs in rural or extensively natural environments remains unknown. *Culex p. pipiens* may also play a role in WNV transmission in MS since there is evidence it occurs in the extreme northern part of the state (Kothera et al. 2009) and it was shown to be the primary vector in the northeastern states (Lanciotti et al. 1999, Andreadis et al. 2004). These two species crossbreed to

create a broad stable hybrid zone in Mississippi (Kothera et al. 2009). This hybrid species has been shown to be an efficient vector of WNV in a laboratory setting (Ciota et al. 2013) and may serve as a bridge vector (Diaz-Badillo et al. 2011).

1.5 WNV surveillance and testing

An important component of arbovirus surveillance is monitoring various arthropod vectors for presence of pathogens (Goddard 2013). Since arboviruses pose a significant threat to public health, it is important to implement sampling and testing of vectors for early arboviral detection in order to perform adequate intervention, prevention, and vector control strategies, that in combination, will interrupt both enzootic and accidental transmission cycles (Gu and Novak 2004). Arbovirus detection in mosquitoes is a critical component in any public health surveillance and control program, and may provide lead time or advance warning of impending human cases. The purpose of this work is to guide mosquito control activities in a more strategic and cost-effective manner to reduce human infection.

1.5.1 VectorTest® West Nile Virus antigen panel assay

The VectorTest® is a rapid immunochromatographic assay (dip-stick test) intended for the qualitative determination of WNV antigens in infected mosquitoes. It is relatively inexpensive and can be utilized for virus detection in mosquitoes using only minimal laboratory equipment. While PCR-based testing methods are the industry standard for virus identification, the availability of a simple, stable, sensitive and rapid diagnostic test, such as VectorTest®, can make arboviral surveillance more cost-effective to state and local surveillance programs (Lampman et al. 2006 and VectorTest 2012).

1.6 Environmental risk factors

It is not uncommon for Mississippi to be one of, if not the very first, state in the U.S. to report human WNV each year. Overall, Mississippi has one of the highest WNV incidence rates in the southeast U.S. (Figure 1.5) and generally throughout the nation (Lindsey et al. 2008, CDC 2013). WNV has had a significant impact on Mississippi as a cause of morbidity and mortality in humans over the past decade. Whether or not this higher rate of WNV infection in Mississippi is due to the state's high poverty rate or (predominantly) outdoor occupations is unknown. More information is needed to determine what roles environmental (including man-made), behavioral, socioeconomic, and educational factors might play as they relate to human WNV infection in Mississippi. Knowing which factors or combinations of factors are most associated with human cases is necessary when it comes to developing and targeting public health messages, intervention methods, and public health policies.

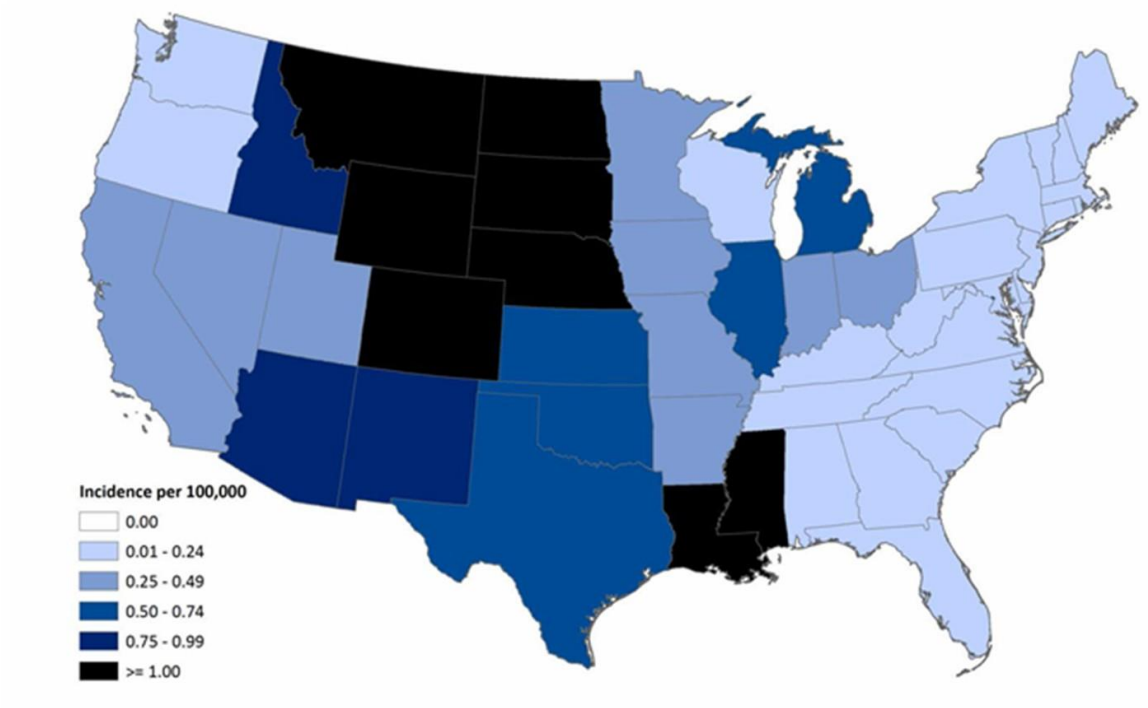

Figure 1.5 Average annual incidence of West Nile virus neuroinvasive disease reported to CDC by state, 1999-2013.

Source: Centers for Disease Control and Prevention.

1.7 Purpose of this study

This dissertation originates from the ongoing need to better understand WNV epidemiology and ecology in Mississippi. Continued research on these aspects of WNV will lead to quicker and more effective public health interventions to prevent and manage the disease.

1.8 Dissertation Objectives

1. Examine the ecology and habitats of the primary WNV vector, *Cx. quinquefasciatus* in Mississippi. More specifically, to determine abundance and prevalence of the primary WNV vector in a rural area of Mississippi.

2. Evaluate the use of the commercial wicking assay, Vectortest®, for WNV detection in *Cx. quinquefasciatus* mosquitoes in Mississippi.

3. Conduct epidemiological and ecological surveys of human WNV infections in Mississippi to identify key factors associated with human disease.

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CHAPTER II

ABUNDANCE AND DIVERSITY OF MOSQUITO SPECIES COLLECTED FROM A RURAL AREA OF CENTRAL MISSISSIPPI: IMPLICATIONS FOR WEST NILE VIRUS TRANSMISSION IN MISSISSIPPI

2.1 Abstract

To determine abundance and seasonality of potential WNV mosquito vectors in a forested area of central Mississippi, mosquitoes were collected weekly from a wildlife management area located approximately 10 miles from a local urban area known to have numerous human WNV cases. I was particularly interested in the presence or absence of *Culex quinquefasciatus*, the primary vector of WNV in Mississippi, although other *Culex* species were assayed. Two CDC light traps baited with CO2 were set once a week from 2005 through 2006 in the Pearl River Wildlife Management Area (PRWMA) which consists of 6,925 acres primarily composed of bottomland hardwood forest with wetland areas. Traps were placed midafternoon and picked up the following morning. A total of 199,222 mosquitoes were collected during the two-year study. No *Cx. quinquefasciatus* were collected throughout the entire study, although other health department surveys have indicated they are abundant just a few miles away. As for other potential WNV vectors, 1,325 (0.6%) *Cx. nigripalpus*, 1,804 (0.9%) *Cx. restuans*, and 6,076 (3.1%) *Cx. salinarius* were collected in the PRWMA over the two-year period. These data suggest

that *Cx. quinquefasciatus* is not usually found in remote forested environments, but is more associated with human habitation.

2.2 Introduction

West Nile Virus was first discovered in the Western Hemisphere in 1999 in New York City, where there were a total of 59 cases reported and seven deaths (Asnis et al. 2000, Mostashari et al. 2001). By 2002, the virus had spread to 45 of the 48 contiguous states, two provinces in Canada, and six states in Mexico (Estrada-Franco et al. 2003). Since its introduction, WNV has been detected in 326 different species of birds and has been associated with, and in some cases caused, deaths in several mammal species including horses, squirrels, dogs, cats, sheep, domestic rabbits, eastern chipmunks, bats, striped skunks, raccoons, harbor seals and at least one captive killer whale (St. Leger et al. 2011, Root 2013). Other evidence suggests that the American alligator may be able to transmit WNV to other alligators and possibly their human handlers (Kienk et al. 2004).

WNV was first documented in Mississippi in humans in July of 2002 (CDC 2002). By the end of that year, Mississippi had a total of 192 WNV cases reported with 162 (84%) of those resulting in the more serious neuroinvasive form of encephalitis; there were 12 deaths (CDC 2002). In the decade since its introduction into Mississippi in 2002, WNV has continued to persist statewide. There has not been a leveling off of WNV cases, but rather a consistent cyclic pattern evidenced by up and down years (Fig. 1). In 2012, Mississippi experienced its highest number of human WNV cases in a single year with a total of 251 cases and 5 deaths (MSDH 2014).

As of 2009, 64 species of mosquitoes have tested positive for WNV in the U.S. (CDC 2014). Of those 64 species, 42 are known to occur in Mississippi (Goddard et al. 2009), but only a few are competent vectors of WNV. As far as is known, ornithophilic *Culex* species, specifically *Culex quinquefasciatus* Say, *Cx. pipiens* L., *Cx. tarsalis* Coquillet, and *Cx salinarius* Coquillet are the most important vectors of WNV in the U.S. (Table 2.1) (Andreadis et al. 2001, Bernard et al. 2001, Blackmore et al. 2003). Both *Cx. quinquefasciatus* and *Cx. pipiens* belong to the *Culex pipiens* complex that occurs in the Mississippi River Basin. The complex is made up of four primary taxa: *Cx. p. pipiens* form *pipiens* L., *Cx. p. quinquefasciatus* Say, hybrids between *Cx. p. pipiens* f. *pipiens*, and *Cx. p. quinquefasciatus*, and *Cx. p. pipiens* form *molestus* Forskål (Kothera et al. 2009, Mutebi and Savage 2009, Kothera et al. 2010).

In Mississippi, *Culex p. quinquefasciatus* is the primary vector for WNV (Rutledge et al. 2003, Godsey et al. 2005, Hayes et al. 2005). *Culex p. pipiens* may also play a role in WNV transmission in the extreme northern part of the state (Kothera et al. 2009). This species is the primary vector in the northeastern states (Lanciotti et al. 1999, Andreadis et al. 2004). These two species crossbreed to create a broad stable hybrid zone in Mississippi (Kothera et al. 2009). This hybrid species has been shown to be an efficient vector of WNV in the laboratory (Ciota et al. 2013) and may serve as a bridge vector in nature (Diaz-Badillo et al. 2011).

Although *Culex nigripalpus* may play a limited role in WNV transmission in Mississippi, *Cx. salinarius* and particularly *Cx. restuans* are more likely enzootic vectors of WNV in the State (Table 2.1). Blood meal analysis evidence suggests that

Cx. salinarius may also play a role as a possible bridge vector to humans (Apperson et al. 2004, Gingrich and Williams 2005, Turell et al. 2005).

Table 2.1 Potential mosquito vectors of WNV in Mississippi.

Species	When active*	Birds	Mammals	Humans
Cx. quinquefasciatus	May -Sept	Mostly	Occasionally	Readily
Cx. pipiens	May-Sept	Mostly	Occasionally	Readily
Cx. tarsalis	Aug-Sept	Readily	Occasionally	Occasionally
Cx. salinarius	Apr-Nov	Occasionally	Mostly	Readily
Cx. nigripalpus	Aug-Nov	Readily	Readily	Occasionally
Cx. restuans	Feb-Jun	Mostly	Occasionally	Rarely

*Mississippi seasonality data only.

The purpose of this study was to determine the various mosquito species present in a rural setting (a wildlife area) in Mississippi, and in particular, any potential West Nile virus vectors such as *Culex quinquefasciatus*, their seasonal activity, and relative abundance.

2.3 Materials and Methods

2.3.1 Collection area

The Pearl River Wildlife Management Area (PRWMA) is one of 48 wildlife refuges maintained by the Mississippi Department of Wildlife, Fisheries and Parks. The PRWMA is located on the boundary of Madison and Rankin counties in central Mississippi approximately 15 miles northeast of the capitol city of Jackson and is in close proximity to a heavily urbanized area (Madison, MS) (Figure 2.1). The wildlife area comprises approximately 6,925 acres and adjoins the north end of the Ross Barnett reservoir where it unites with the Pearl River. The Ross Barnett reservoir is a 33,000 acre man-made lake created by the impounding of the Pearl River and serves as the drinking

water source for the Jackson metro area. PRWMA is characterized by temperate weather conditions representative of all but the coastal and most northeastern areas of the state and comprises a myriad of non-urban habitats such as low lying hardwood bottomland forest, fresh water marshes, cypress swamps, pine forests, and open food plots purposely planted for wildlife management. The topography varies from flat to gently rolling hills and consists principally of acidic sandy silt loam and clay/clay loam. Approximately 20% of the PRWMA is designated as a waterfowl refuge and is flooded annually as part of the waterfowl management program.

Figure 2.1 Map of Pearl River Wildlife Management Area in relation to urban area hotspots (particularly areas surrounding the airport).

2.3.2 Collection method

Battery-powered Centers for Disease Control (CDC) light traps baited with CO_2 (Figures A.1 and A.2) were used in this study to systematically assess adult mosquito populations, as opposed to New Jersey light traps which require a fixed source of electricity. Studies have shown that collections are more diverse when using baited CDC light traps as compared to New Jersey light traps (Harden and Poolson 1969). Further, some species are not attracted to light alone and require a CO_2 source (Silver 2008).

Traps were placed in the youth and handicapped hunting areas adjacent to the waterfowl refuge portion of the WMA which is approximately 1,200 acres. This was done primarily for safety purposes since this area has more restrictive hunting regulations than the rest of the WMA and is seldom visited by hunters. During the winter months, traps were deliberately set on the warmest days in order to collect the most mosquitoes possible.

The two traps were placed approximately 0.5 mi from each other (Figure 2.2) and were baited with 2.5 lbs of dry ice pellets each. Traps were placed one day per week around 2:00 p.m. retrieved 9:00 a.m. the following day. Mosquitoes from both traps were combined for analysis. Samples were taken to the Mississippi State Department of Health Public Health Laboratory (MSDH PHL) where they were placed in a -20°F freezer for a minimum of 2 hours to kill any live mosquitoes prior to identification and testing. Adult mosquitoes were identified using illustrations in Carpenter and LaCasse (Carpenter & LaCasse, 1955), the keys of Darsie and Ward (R. Darsie, Jr & Ward, 2005), Slaff and Apperson (Slaff & Apperson, 1989), and Darsie and Morris (R. F. Darsie & Morris, 2003). Representatives of all species collected were sent to Dr. Bruce Harrison

for confirmation. Voucher specimens have been deposited in the Mississippi Entomological Museum, Mississippi State University.

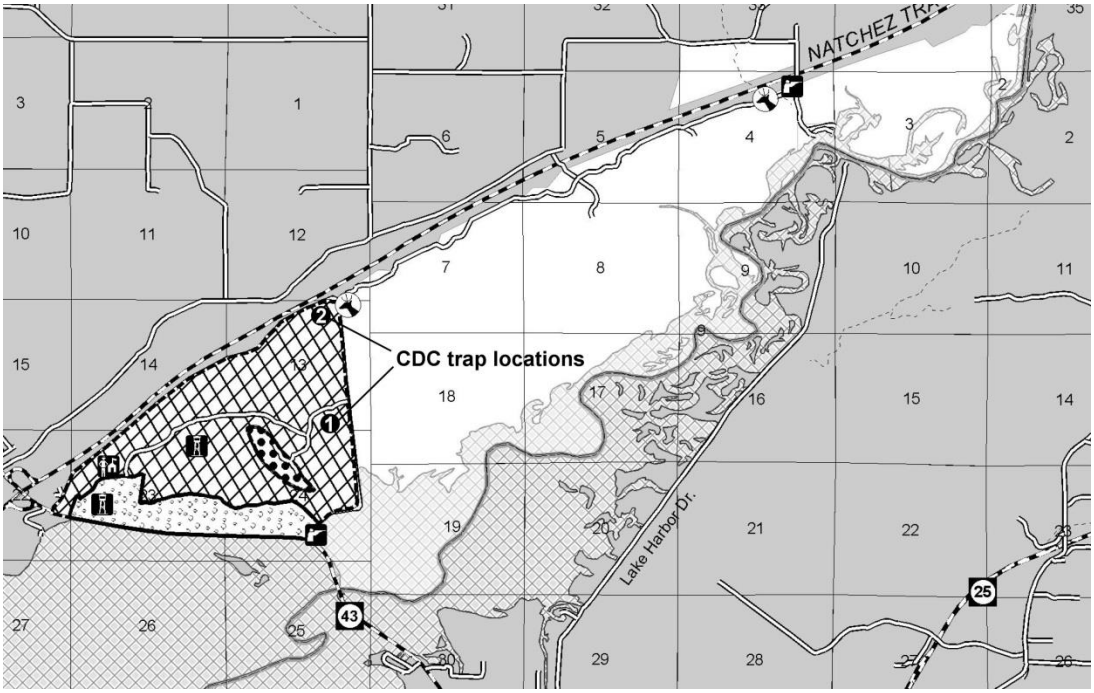

Figure 2.2 Two CDC light trap locations within the Pearl River Wildlife Management Area.

The hatch-mark and bold dotted areas are reserved for youth and handicap hunting respectively. The hollow dotted area below hatch-mark represents the waterfowl refuge. Map: Mississippi Department of Wildlife, Fisheries, and parks.

2.4 Results and Discussion

A total of 199,212 mosquitoes were collected during the two-year study with 72,677 collected in 2005 and 126,535 collected in 2006. The difference in total number of mosquitoes collected in 2005 versus 2006 is due to a four-week interruption in data collection in the aftermath of Hurricane Katrina. Access to the area was prohibited due to multiple fallen trees that took weeks to remove.

A total of 27 species representing nine genera was collected and are listed in Table 2.2. The number of species collected per genus is shown in Figure 2.3. *Anopheles crucians* and *Coquilletidia perturbans* were the dominant species collected throughout the entire study. Both species combined represented approximately 72% (142,947) of the entire collection with 40% (79,512) being *An. crucians* and 32% (63,435) being *Cq. perturbans*. The next most abundant species collected were *Culex erraticus, Aedes vexans, Culex salinarius,* and *Psorophora ferox,* respectively. Seasonality and abundance of these primary species collected are represented in Figure 2.4.

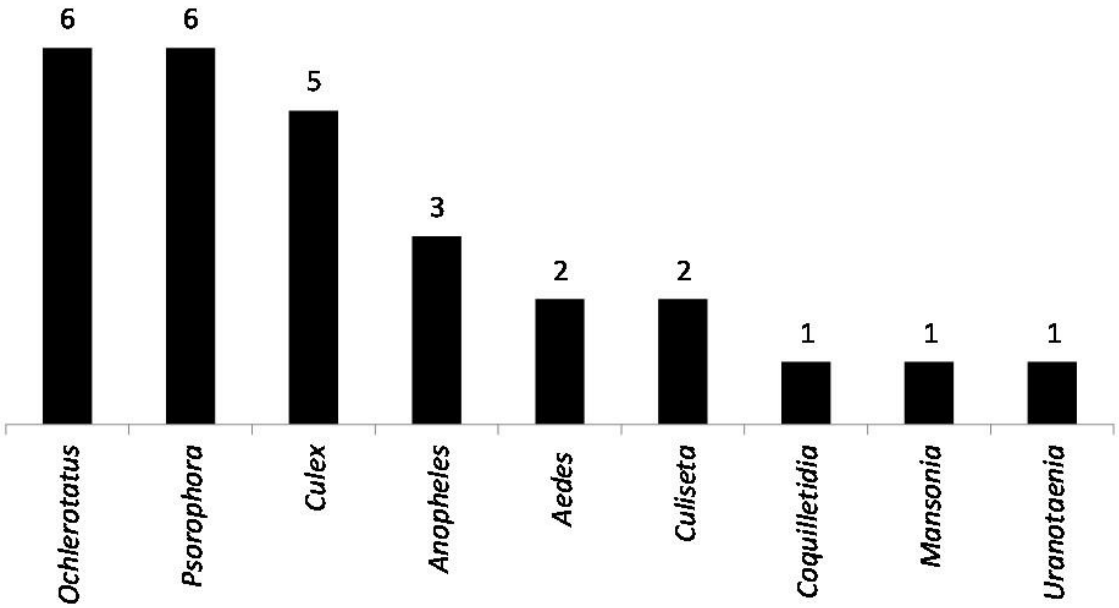

Figure 2.3 Number of mosquito species representing each genus collected in the Pearl River Wildlife Management Area, 2005-2006.

Table 2.2 List of all mosquito species collected from Pearl River Wildlife Management Area 2005-2006.

Species Collected	2005	2006	Total	Peak Activity	Total Months Occurring
Anopheles crucians	31690	47822	79512	Apr-Aug	12
Coquilittidia perturbans	20341	43094	63425	May-Sept	8
Culex erraticus	5862	13622	19484	May-Aug	11
Aedes vexans	1140	8803	9943	Apr-Jun, Nov-Dec	12
Culex salinarius	1311	4764	6075	May-Jul	12
Psorophora ferox	5018	888	5906	May-Oct	9
Uranotaenia sapphirina	2003	1965	3968	Jun-Aug	11
Anopheles quadrimaculatus	1530	2418	3948	May-Sep	10
Culex restuans	138	1666	1804	Mar-May	7
Ochlerotatus atlanticus/tormentor	1397	67	1464	Jun- Jul, Oct	7
Culex nigripalpus	554	771	1325	Sep-Oct	4
Ochlerotatus fulvus pallens	975	248	1223	Jun-Jul	5
Psorophora howardii	280	69	349	May-Jul	6
Culiseta inornata	74	182	256	Jan-Mar, Oct-Dec	7
Ochlerotatus canadensis	145	73	218	Apr-May	4
Ochlerotatus dupreei	111	0	111	May, Oct	7
Mansonia titillans	32	44	76	Sep-Oct	4
Anopheles punctipennis	27	19	46	Jan-Mar, Sep-Oct	5
Ochlerotatus sticticus	17	0	17	Apr-May	2
Ochlerotatus triseriatus	11	3	14	Apr-Oct	7
Psorophora mathesoni	12	0	12	Jun-Jul	5
Psorophora columbiae	1	8	9	Aug-Sep	2
Culex coronator	1	7	8	Aug	3
Aedes albopictus	4	0	4	Oct	1
Culiseta melanura	3	0	3	Apr-Jun	4
Psorophora ciliata	0	1	1	Aug	1
Psorophora cyanescens	0	1	1	May	1
Total	**72677**	**126535**	**199212**		

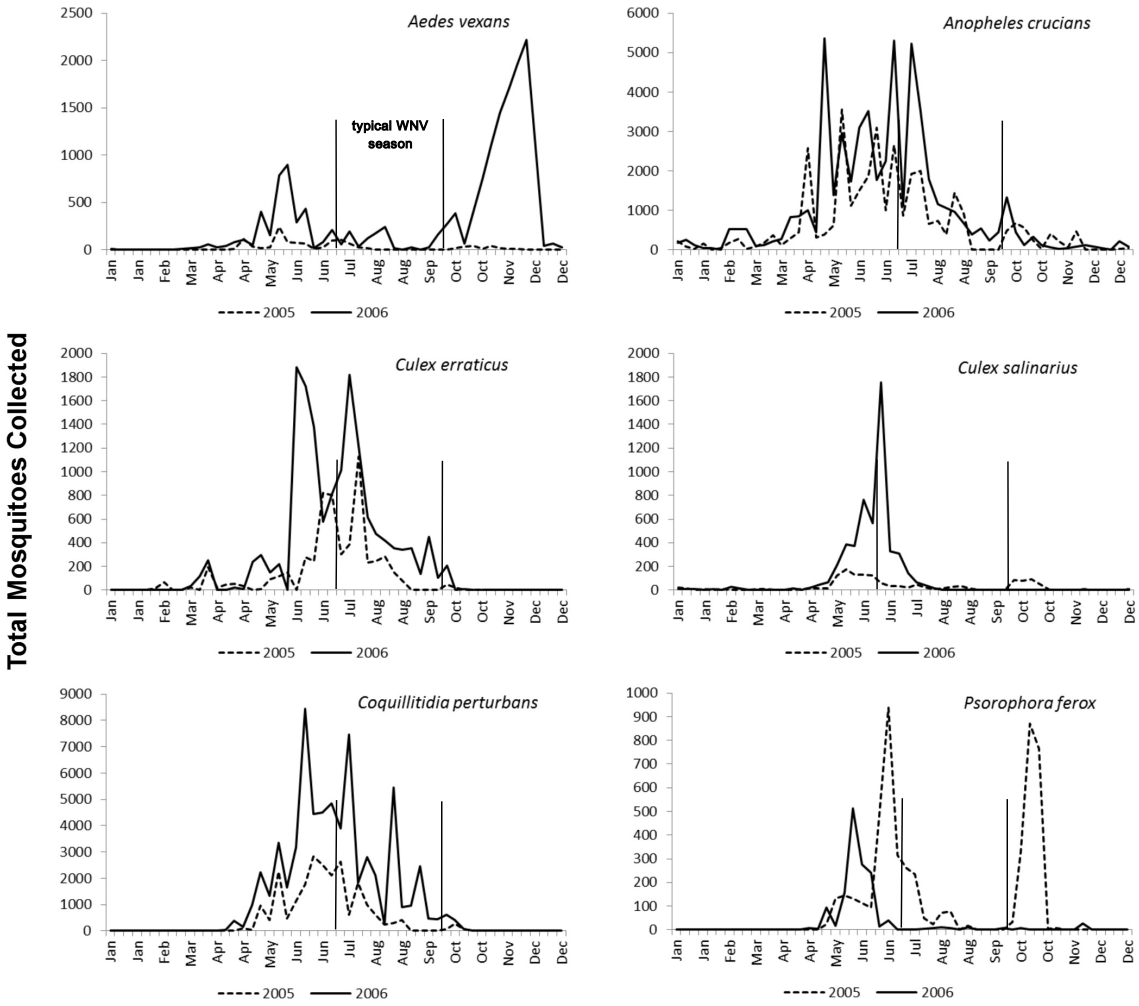

Figure 2.4 Seasonality and abundance of primary species collected in PRWMA. Vertical lines represent typical WNV season in Mississippi.

Weather data from the nearest weather station (Jackson International Airport 32.3205°, -90.0777°, Elevation: 331ft) showed that the mean low mean temperature in January for both years was 41° F (2005) and 40.3° F (2006) and mean high in August for both years was 93.7° F (2005) and 95° F (2006). Annual rainfall was 52.05" and 51.23" for 2005 and 2006, respectively.

Of the approximately 61 species of mosquito species known to occur in Mississippi (Varnado et al. 2012), 27 were collected in the PRWMA during our two-year study. Twelve genera of mosquitoes have been previously identified in Mississippi (Peterson and Smith 1945, Michener 1947, Goddard et al. 2007); nine were collected in this study. *Orthopodomyia signifera* has been previously collected in PRWMA (Oct. 2004), however, none was collected in this two-year study.

Three species (*An. crucians, Ae. vexans, and Cx. salinarius*) were active throughout all twelve months of the year, *Culex erraticus* and *Ur. sapphirina* were active eleven months of the year, and *An. quadrimaculatus* ten months. Most species, however, were generally active for a few months out of the year during late spring or summer months. *Culiseta inornata* is the only mosquito species in Mississippi that is exclusively active during winter. *Anopheles crucians* and *Ae. vexans* can also be active during the winter months, particularly on unseasonably warm days (Table 2.2); however, as with most species, their greatest numbers are generally seen in the spring and summer months.

The majority of mosquitoes collected during this study were permanent water species, that is, species which breed in swamps, lakes and ponds. This includes *Anopheles* spp., certain *Culex* spp. and *Coquillitedia* sp. This could have been due to seasonal fluctuations in rainfall during the study (Figure 2.5). The marshy areas of the PRWMA are inundated with cattails and sedges which are periodically thinned by controlled burns. Since *Cq. perturbans* are associated with cattails it is expected that their numbers would be quite high. Elevated numbers of *An. crucians* were likely due to the routine controlled flooding and draining of the waterfowl refuge. Induced flooding in the PRWMA typically occurs around the first of October each year. As a result, mid-

October of both years experienced a peak emergence of the flood water species *Ps. ferox* (2005) and *Ae. vexans* (2006) (Figure 2.4).

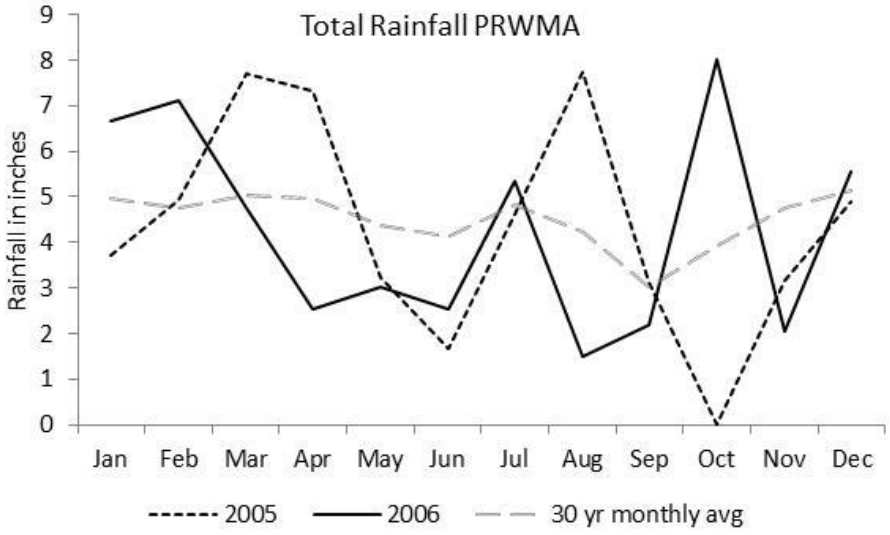

Figure 2.5 Monthly rainfall in the Pearl River Wildlife Management Area, 2005-2006.

While the abundance of *Anopheles crucians* and *Coquilletidia perturbans* was not surprising, there were a few species whose collection was unexpected. *Aedes albopictus* is typically found in urban areas in and around tire piles and other containers. Two specimens each were collected on 4-Oct-2005 and 19-Oct-2005 for a total of four specimens. It is likely the four specimens emerged from a tire found lying in the bushes approximately 100 feet from the trap. Larval dipping of the tire indicated *Ae. albopictus* was the only species present. The second unexpected species collected was *Culex coronator*. This species was first collected in Mississippi in the fall of 2004 in Copiah County (Varnado et al. 2005, St. Leger et al. 2011). A single specimen was collected in the PRWMA on 11-Aug-2005, which resulted in Madison County being the second

Mississippi county to report *Cx. coronator*. *Cx. coronator* has been shown to be highly susceptible to WNV infection and dissemination in a laboratory setting; however, its ability to transmit WNV in a natural setting is probably limited (Alto et al. 2014).

Other potential WNV vectors collected over the two-year period included, 1,325 (0.6%) *Cx. nigripalpus*, 1,804 (0.9%) *Cx. restuans*, and 6,076 (3.1%) *Cx. salinarius.* These species are considered potential, but not likely, vectors of WNV in Mississippi, in part due to their host feeding preferences (Table 2.1) (Kilpatrick et al. 2005, Turell et al. 2005). Also, the seasonal activity of these other potential vectors, with the exception of *Cx. nigripalpus*, does not match the seasonality of WNV human cases in Mississippi (Figures A.3-A.6). Interestingly, not one *Cx. quinquefasciatus* was collected over the two-year period, which has been reported as the primary vector for WNV in MS. These data suggest that *Cx. quinquefasciatus* is not usually found in remote forested environments, but is more highly associated with human habitation.

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CHAPTER III

USE OF THE VECTORTEST® FOR ADVANCED WARNING OF

HUMAN WEST NILE VIRUS IN MISSISSIPPI

3.1 Abstract

West Nile Virus (WNV) continues to persist in Mississippi, with 2012 being the worst year to date for human infections with a total of 251 reported human cases and 5 deaths. Public health officials are keenly interested in ways to detect WNV in advance of human transmission in their jurisdictions, with the ultimate goal of implementing appropriate and timely mosquito control measures in affected areas. Previous studies have utilized landscape ecology, weather and demographic data to try to predict WNV activity. Mosquito numbers and infection rates also may be used in modeling and prediction efforts. The VectorTest® has been demonstrated in several research and validation studies to be a specific and effective immunochromatographic assay (dip-stick test) which can provide rapid WNV threat assessment for mosquito control personnel. This study evaluated if, and to what extent, mosquito testing using the VectorTest® could provide *advanced* warning of impending human WNV cases in a specific area. A total of 40,312 female *Culex quinquefasciatus* mosquitoes was collected by gravid traps in 9 areas in Mississippi during 2013 and 8 areas during 2014. These mosquitoes were subsequently tested by VectorTest® and these data were compared with human WNV cases date of onset to determine predictive value of mosquito testing for WNV activity.

Both years, and in all collection areas, positive mosquito pools appeared before the vast majority (87.2%) of reported human cases. Overall, 73% of human WNV cases occurring in our study sites, there was an average advanced warning of 26 days as indicated by positive mosquito collections near the patient's home. This operational health department study, although somewhat limited, reveals that mosquito sampling and testing may inform public health and mosquito control personnel of WNV activity in an area and impending human cases.

3.2 Introduction

West Nile virus (WNV) is a mosquito-borne, enveloped single-stranded, positive-sense RNA virus belonging to the Flaviviridae family of viruses (Tesh and Solomon 2011). WNV was first discovered in the Western Hemisphere in 1999 in New York City, NY, U.S.A., where there were a total of 59 cases and 7 deaths (Asnis et al. 2000, Mostashari et al. 2001). In the U.S., WNV has become the leading cause of epidemic meningoencephalitis in humans, however, it is estimated that less than 1% of all WNV infected patients develop the more serious neuroinvasive form of the disease. There are no known specific treatments for WNV and the patient is generally only treated with supportive care. WNV was first documented in Mississippi in humans in July of 2002 (CDC 2002) and by the end of 2002, Mississippi had a total of 192 WNV cases with 162 of those resulting in serious encephalitis; there were 12 deaths (CDC 2002). In the decade since its introduction into Mississippi in 2002, WNV has continued to persist statewide. The year 2012 was the tenth anniversary of the introduction of WNV in Mississippi and proved to be the worst year for human infections with a total of 251 human cases and 5 deaths (MSDH 2014) (Figure 3.1).

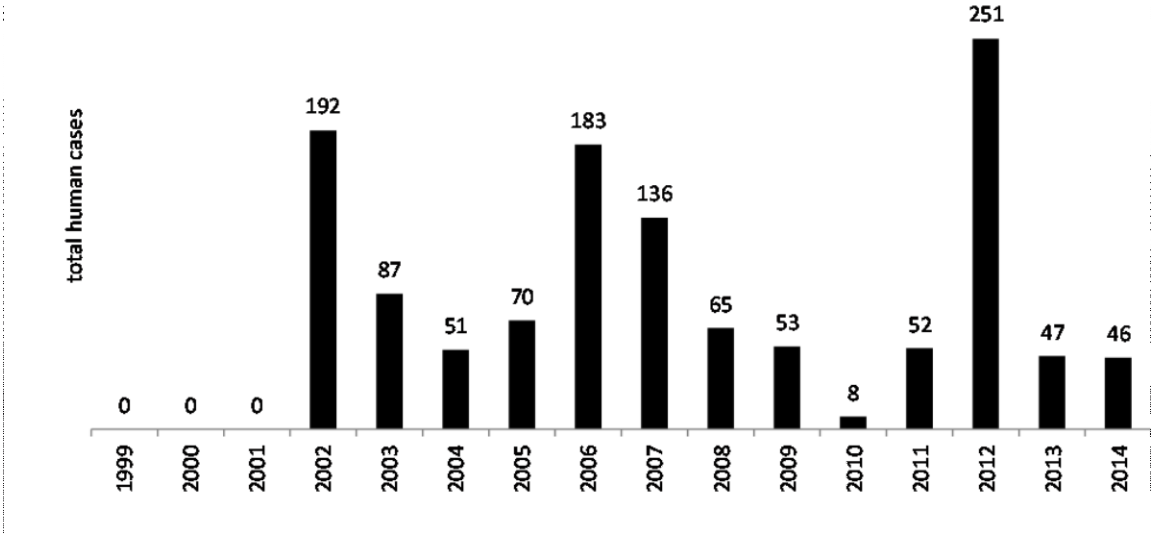

Figure 3.1 Mississippi human West Nile virus cases, 1999-2014.

Public health officials are keenly interested in ways to detect WNV in mosquitoes and sentinel animals, with the ultimate goal of implementing appropriate and timely mosquito control in affected areas (Gu and Novak 2004, Goddard 2013). Some studies have utilized landscape ecology, weather and demographic data to try to predict WNV activity (Gu et al. 2008, Young et al. 2013, Manore et al. 2014). Mosquito numbers and WNV infection rates also may be used in WNV modeling and prediction efforts. There are several methods available for testing mosquitoes for WNV including reverse transcription polymerase chain reaction (RT-PCR), various plaque assays, and viral antigen assays. The VectorTest® (VecTOR Test Systems, Inc., Thousand Oaks, CA) is a rapid immunochromatographic assay (dip-stick test) intended for the qualitative determination of WNV antigens in infected mosquitoes. While PCR-based testing methods are the industry standard for virus identification, the availability of a simple, stable, sensitive and rapid diagnostic test such as the VectorTest® makes arboviral

surveillance more cost-effective to state and local surveillance programs. Although the VectorTest® (previously known as VecTest®) might miss some positives as compared to PCR assays, it has been shown to be highly specific and an effective rapid threat assessment tool for mosquito control personnel (Burkhalter et al. 2006, Turell et al. 2011). In fact, one study demonstrated that the VectorTest® for WNV detection was .98 accurate, .97 sensitive, and 1.0 specific (Chiles et al. 2004), and another showed that it detected 100% of positive mosquito pools (Sutherland and Nasci 2007).

Several studies have attempted to link mosquito surveillance data with human WNV infections (Liu et al. 2009, Ginsberg et al. 2010, Kilpatrick and Pape 2013). The best early season predictors of WNV activity have been found to be 1) early date of first positive pool, 2) low absolute numbers of mosquitoes in July, and 3) low numbers of mosquito species (diversity) in July (Ginsberg et al. 2010). The latter two are associated with drought conditions which have been shown to be inversely related to WNV infection, i.e., heavy rainfall inhibits WNV infectivity and lack of rain promotes it (Goddard 2008). Studies have also shown that the number of WNV positive mosquitoes in an area (within the last 30 days) is a significant predictor of human infection risk (Liu et al. 2009) and that standardized mosquito surveillance and testing provides strong predictive power to signal human WNV infection several weeks in advance (Kulasekera et al. 2001, Kilpatrick and Pape 2013). In addition, minimum infection rates (MIR)[1] and vector mosquito abundance can be combined into a vector index which is a good indicator of human WNV risk, a method advocated by the Centers for Disease Control (Jones et al. 2011, Kwan et al. 2012, Chung et al. 2013). The purpose of this study was

[1]According to the CDC, the MIR is the number of infected mosquitoes per 1,000 tested (see section 3.5).

to determine if, and to what extent, environmental health personnel can use mosquito testing (VectorTest® in this case) to acquire advanced warning of impending human WNV cases in a specific area.

3.3 Materials and Methods

3.3.1 Collection sites

Nine areas throughout Mississippi were selected for mosquito sampling based on previous Health Department surveillance and research. Three of the areas consisted of more than one town/city in one geographic location; all other areas were cities by themselves (Figure 3.2). The Golden Triangle collection area included the towns of West Point, Starkville, Columbus, and Louisville. The Jackson Metro area included Cities of Jackson, Pearl, Brandon, and Canton. The Biloxi/Gulfport area included the parts of Harrison County covered by these two cities. Five of the locations are known human WNV hotspots based on historical health department data and four of them historically exhibited little annual human WNV activity. All collections were made in urban areas known to potentially harbor *Cx. quinquefasciatus* mosquitoes, and thus were considered favorable for WNV activity.

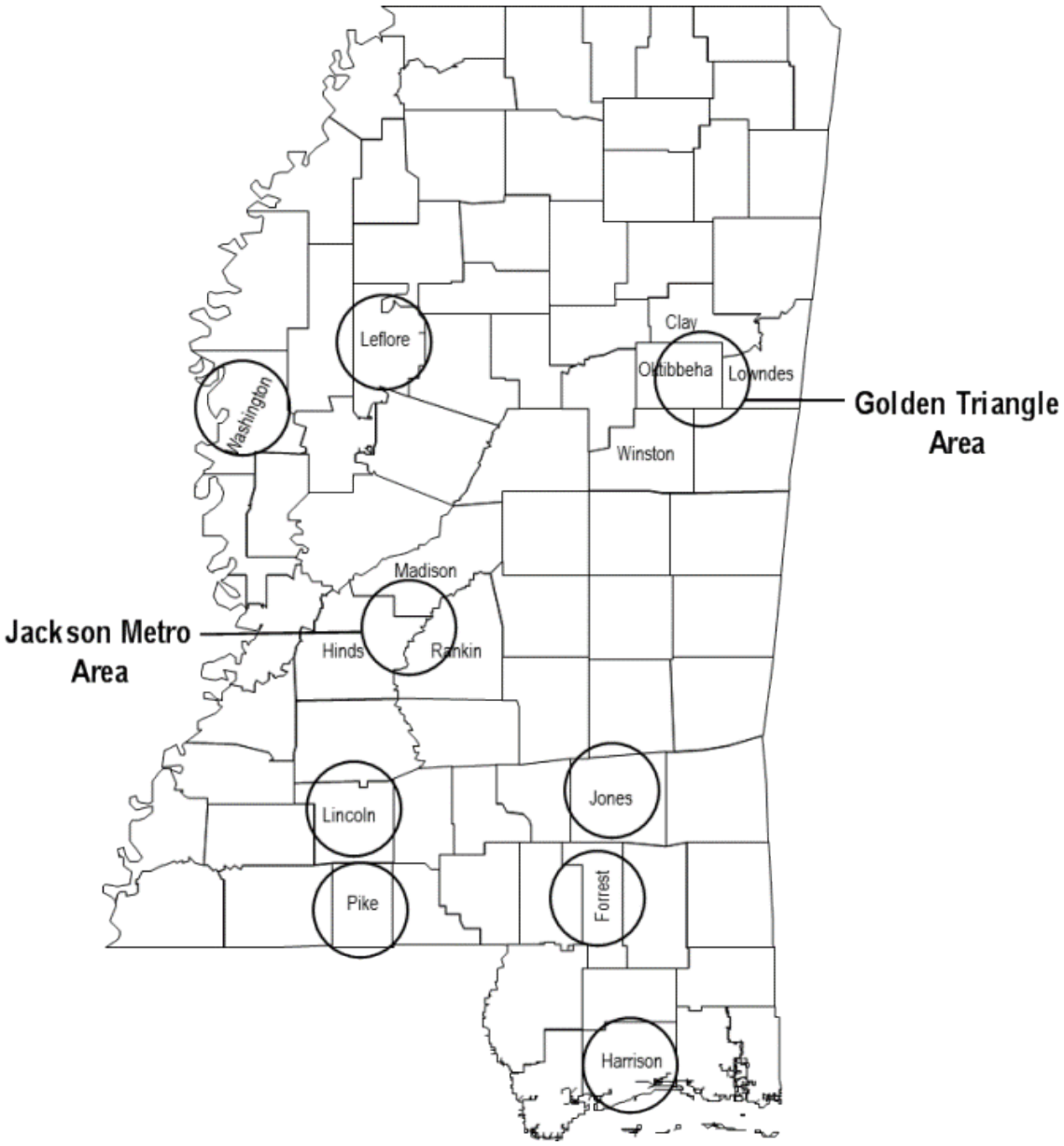

Figure 3.2 Nine collection sites within Mississippi where gravid trapping was conducted for WNV mosquito testing.

3.3.2 Mosquito collections

Local county mosquito control staff, health department personnel, and health department interns were tasked with operating CDC gravid traps (John W. Hock model 1712) (Appendix Figure B.1) at the selected sites from approximately June 1st until September 15th each year. Gravid traps were used in this study because they target the mosquito vectors of WNV, and specifically, previously blood-fed mosquitoes. Note: Sampling dates varied somewhat due to health department administrative and budget issues. All personnel involved in trapping received appropriate training prior to initiation of the project. Gravid traps were used because they primarily attract female *Cx. quinquefasciatus* mosquitoes which oviposit their eggs in highly organic water (e.g., containers with decaying leaves and septic ditches). Traps were set weekly at each site in late afternoon and retrieved the following morning, unless prohibited by inclement weather. Each trap was powered by one 6 V, 10 Amp rechargeable gelled-electrolyte battery. Traps were baited with a fish-oil emulsion mixture containing approximately three ounces of fish-oil emulsion to one gallon of water. Once the net was retrieved from the trap, mosquitoes were sorted into pools of no more than 50 female mosquitoes each. A collection is defined here as the total amount of female mosquitoes collected in one trap night which may be subdivided into smaller groups called batches or pools for testing. In this study, due to financial constraints, no less than 10 mosquitoes were included in a pool for testing. Mosquito pools were then transported or shipped to the state public health laboratory for WNV testing.

3.4 VectorTest® procedure and quality assurance testing

At the health department, mosquito identifications were confirmed and then pools were tested by VectorTest® according to manufacturer instructions (Figure B.2). This included macerating the mosquitoes in a buffer solution, centrifuging the slurry, obtaining a supernatant and then testing the resulting fluid with the test strips. Test strips were read within 30 minutes of the assay. Any strips with indistinct bands were classified as presumptive positive samples. For outside quality assurance, all mosquito pools that tested positive, as well as the presumptive samples were sent to CDC, Division of Vector-Borne Diseases, Arboviral Diseases Branch in Fort Collins, CO for follow-up testing and confirmation with RT-PCR using previously described methods (Ryan et al. 2003, Burkhalter et al. 2006).

3.5 Calculation of minimum infection rate (MIR)

Estimates of minimum infection rate (MIR) are usually presented as the number of infected mosquitoes per 1,000 tested. For example, in the simplest estimate, MIR is calculated as ([number of positive pools / total specimens tested] x 1000), with the data representing a single species or species group collected over a time period and geographic area relevant to the goals of the particular surveillance program. MIR is based on an assumption that a positive pool contains only one infected mosquito, an assumption that may be invalid when infection rates are high, as has been observed during West Nile virus epidemics (CDC 2013).

At the county level or below, weekly tracking of MIR can provide important predictive indicators of transmission activity levels associated with elevated human risk.

3.6 WNV human case data

Human WNV cases were determined using the Mississippi State Department of Health (MSDH) EpiTracks system. These included clinical cases confirmed by the MSDH Public Health Laboratory and/or the Centers for Disease Control, private reference laboratories, and blood banks. No personal information was collected in this analysis and cases were plotted on maps only to the nearest cross street. Date of onset was defined as the initial date the patient recalled symptoms (not date of doctor visit).

3.7 Results and Discussion

A total of 40,312 (16,259 in 2013, 24,053 in 2014) *Cx. quinquefasciatus* was collected in the nine sites over the two-year period with an average of 72.6 per trap (77.3 in 2013 and 66.91 in 2014) ranging from 5 to 900 in 2013 and 10 to 900 in 2014 (Figures B.3 and B.4). During 2014, no collections were made from Greenwood due to personnel issues; therefore there were only 8 sites that year. As for mosquito infectivity with WNV, we know from previous studies that the VectorTest® can detect 65-100% of positive pools (Burkhalter et al. 2006, Sutherland and Nasci 2007), therefore I am confident that the screening method was able to detect infected mosquitoes in the study sites. The overall MIR over the 2-year period ranged from 0.0 to 9.9/1,000 with Hattiesburg having the highest MIR and Biloxi/Gulfport, Brookhaven, and the Golden Triangle areas having the lowest (Figures B.3 and B.4). The low MIR of *Cx. quinquefasciatus* in Brookhaven during 2013 is likely due to lack of trap data, but interestingly, the Golden Triangle area had a zero MIR despite 22 collections. The low absolute numbers and MIR on the Mississippi Coast is likely due to the presence of a well-run county-wide integrated mosquito control program in that area.

There were 18 confirmed human WNV cases in the 9 collection areas during 2013, and 21 cases in 8 sites in 2014. Both years, and in all collection areas, positive mosquito pools appeared before the vast majority (87.2%) of reported human cases (Figure 3.3). In two instances, there human WNV cases in a study site without prior positive mosquito collections – Brookhaven, MS during 2013 and Biloxi/Gulfport during 2014. Thirty-seven of the WNV cases occurred within 4 miles of any gravid trap (and our analysis is based on those cases). The 4 mile distance between WNV cases and nearby gravid traps was chosen based upon average acreage covered in typical mosquito spray zones.

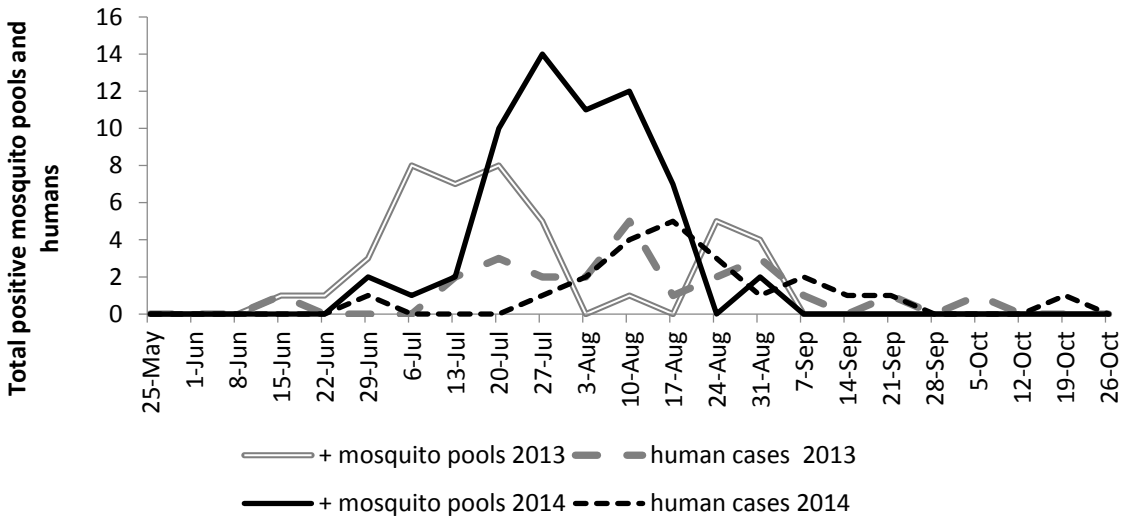

Figure 3.3 Overall pattern of dates of mosquito infection and human WNV symptom onset, all sites combined 2013-2014, demonstrating that mosquitoes become infected well before human cases occur.

Overall, in 27/37 (73.0%) human WNV cases occurring in our study site, there was an average advanced warning of 26 days (range 11 to 53 days) as indicated by

positive mosquito collections near the patient's home. This of course, assumes that the patient contracted WNV at or near their home. There was a trend of higher MIRs leading to better chances of predicting human WNV cases in an area (Figures B.3 and B.4).

Outside confirmation of our results by the CDC showed that 34/36 (94.4%) of VectorTest® positive samples were also WNV positive when re-tested by RT-PCR testing (Figure B.5). This means that 2 samples were false positive by VectorTest®. The cause of this discrepancy is unknown but could be from human error in reading the test strips. Of the 7 presumed positive samples submitted from 2013, all but 1 was positive by RT-PCR (there appeared to be a faintly visible positive line with the VectorTest®). As for 2014, a total of 63/67 of VectorTest® positive samples was WNV positive when re-tested by RT-PCR. Four samples were considered questionable by RT-PCR, possibly suggesting that, while there may have appeared to be a faint positive line on the VectorTest® strip, there wasn't enough viral RNA to accurately confirm positive for WNV. Of the 5 presumed positive samples submitted from 2014, 3 were positive by RT-PCR and 1 fell into the questionable group by RT-PCR testing. Despite these differences in results from VectorTest® and RT-PCR, the 95% confidence limits for infection rates for the two assays have been shown to have a high degree of overlap (Nasci et al. 2002).

This operational health department study, although somewhat limited, reveals that mosquito sampling and testing (in this case by a commercial dipstick test) may inform public health and mosquito control personnel of WNV activity in an area and impending human cases. In this study, only two times did humans acquire WNV in the study sites without first finding positive mosquitoes in those areas. This, of course, could mean

those patients contracted WNV at a location away from their homes. The lead time before onset of human cases ranged from almost two weeks to two months, giving ample time for appropriate health department interventions such as educational campaigns and mosquito control. Further, our study demonstrates that the relatively inexpensive and less labor intensive products such as the VectorTest® are more than adequate for health departments or mosquito control agencies which might not have access to sophisticated and expensive molecular analysis capability.

3.8 Acknowledgements

This study was funded by a grant from the CDC to the Mississippi State Department of Health, Epidemiology and Laboratory Capacity for Infectious Diseases grant number U50\CCU416826-03. A variety of persons helped operate gravid traps statewide, including students (Claire He, Ethan Woodyard, Alexis Hines, Francis Ezeakacha), a health department environmentalist (Anthony Claytor), and mosquito control personnel (Jerry Sykes and Kris New). Kristy Burkhalter (Centers for Disease Control and Prevention, Ft. Collins, CO) performed RT-PCR on selected samples for quality assurance testing.

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CHAPTER IV

A SURVEY OF ENVIRONMENTAL AND BEHAVIORAL ASPECTS OF HUMAN WEST NILE INFECTIONS IN MISSISSIPPI

4.1 Abstract

In the decade since its introduction into Mississippi, West Nile Virus (WNV) has continued to persist statewide with dozens of cases reported each year. The number of cases varies widely from year to year. The most cases occurring in a single year in Mississippi was 2012 with a total of 251 human cases reported and 5 deaths. A variety of ecological and behavioral factors might contribute to human WNV cases, such as local weather conditions, excessive vector breeding near the home, length of time spent outdoors, and lack of personal protection measures. The purpose of this study was to survey socio-economic, environmental, and behavioral factors in patients known to have contracted WNV disease in Mississippi. A total of 377 WNV patients in Mississippi were sent a survey asking questions about environmental conditions around their homes, their neighboring properties, as well as various behaviors such as mosquito source reduction, time spent outdoors, and repellent use. A total of 134 (36%) questionnaires were returned and analyzed. Results showed that a majority of respondents said they paid attention to mosquito breeding sites around their home and made efforts to eliminate them. This fact might demonstrate that clean-up campaigns and other educational efforts by the health department have been useful. Almost all WNV patients in our study who

lived in non-incorporated areas had onsite wastewater systems (septic tanks and treatment plants), but only 19% reported properly servicing and maintaining these systems. The primary vector of WNV in Mississippi, *Culex pipiens quinquefasciatus*, is known to breed in effluent from failing or poorly functioning on-site wastewater systems, so particular attention needs to be paid to this potential breeding site. Interestingly, we found that Mississippians in WNV endemic areas do not get their information about WNV from brochures, other print materials, or radio spots. Television, newspaper and internet were the primary avenues for patient education concerning information about WNV. Also, we found that amount of time spent outdoors seemed associated with WNV infection. Only one of 134 respondents in this study spent less than 2 hours outdoors one day a week. A more controlled study of outdoor activity by people living in WNV endemic areas is warranted. Paradoxically, the use of personal protection techniques such as repellents did not change from before WNV infection to after. This too, highlights the need for continued targeted educational efforts.

4.2 Introduction

West Nile virus (WNV) is a mosquito-borne *Flavivirus* first discovered in the Western Hemisphere in 1999 in New York City, where a total of 59 cases and seven deaths occurred (Asnis et al. 2000, Mostashari et al. 2001). WNV was first documented in humans in Mississippi during 2002 (CDC 2002) and by the end of the year, the Mississippi State Department of Health (MSDH) reported a total of 192 WNV cases with 162 of those resulting in serious encephalitis; 12 deaths occurred (CDC 2002). In the decade since its introduction into Mississippi, WNV has continued to persist statewide with 2012 exhibiting the most human infections with a total of 251 human cases reported

and 5 deaths (MSDH 2014). The most common signs and symptoms of WNV include fever, headache, myalgia, muscle weakness, gastrointestinal disruption, and in some cases a maculopapular rash on the chest, back, and arms. In the U.S., WNV has become the leading cause of epidemic meningoencephalitis in humans, however, it is estimated that less than 1% of all WNV infected patients develop the neuroinvasive form of the disease (Tesh and Solomon 2011). The irreversible neurological damage resulting from meningoencephalitis, meningitis, or acute flaccid paralysis (AFP) is often severe (DeBiasi and Tyler 2006). Even for patients who recover from the disease, there may be serious and debilitating long-term sequelae such as muscle weakness, memory loss, fatigue, and difficulty concentrating (Patel et al. 2015).

A variety of ecological and behavioral factors might contribute to human WNV cases. Local weather conditions and especially rainfall may cause increases in WNV in a given area (Dohm et al. 2002, Shaman et al. 2003, 2005, Reisen et al. 2006, Soverow et al. 2009, DeGroote et al. 2014). Contrary to what one may think, there is an inverse relationship between rainfall and WNV activity (Monath 1980, Goddard 2008). Excessive vector breeding near the home, amount of time spent outdoors during peak mosquito activity (Aquino et al. 2004), and lack of personal protection measures (Loeb et al. 2005, Gujral et al. 2007) also can lead to WNV cases. Furthermore, there have been efforts with varying degrees of success to use landscape ecology and demographic data to try to predict WNV cases (Rogers et al. 2002, Cooke et al. 2006, Gu et al. 2008, Wimberly et al. 2008, Young et al. 2013, Manore et al. 2014). DeGroote et al. (2014) found that pasture, higher available soil water, and younger, poorer black populations were positively associated with higher WNV occurrence, however, the consensus among

several researchers is that there are multiple WNV transmission dynamics complicating predictive modeling (DeGroote et al. 2008).

In public health vector control campaigns, it is important to understand community residents' understanding of personal protection measures against WNV, as well as their role in reducing local mosquito populations (Olsen et al. 2008, Dowling et al. 2013). Although municipalities may conduct occasional clean-up days for abatement of insect vectors, community sustainability (e.g., local citizen interest and diligence concerning mosquito control) is critical for long term success. Therefore, targeted educational campaigns based upon current knowledge, attitudes, and practices of community residents are critical for prevention and management of vector-borne diseases (Slavinski and Jones 2004).

There have been surveys in several states of the economic effects of WNV (Zohrabian et al. 2004, Barber et al. 2010), personal protective behaviors, perceptions of local mosquito control (pertaining to WNV), and sero-epidemiological factors (Slavinski and Currier 2003, Olsen et al. 2008, Tuiten et al. 2009, Bowman et al. 2014). These studies have shown that people are concerned about WNV and ways to protect themselves. For example, 67% of respondents in a 2004 Mississippi survey said they used repellents to protect themselves from mosquito-borne diseases (Slavinski and Jones 2004). In contrast, no ecological or epidemiological surveys of Mississippi WNV human cases have been conducted. The purpose of this study was to survey socio-economic, environmental, and behavioral factors in patients known to have contracted WNV disease in Mississippi.

4.3 Materials and Methods

4.3.1 Survey

With appropriate IRB approval (MSDH protocol # 011114, Figure C.1) a 47-question survey (Figure C.2) was mailed to 400 (out of 469 total) qualifying patients who had been confirmed positive for WNV during the years 2008-2013. Those initially not qualifying for a questionnaire were either minors (total 14) or deceased (total 55). Patients were identified using the MSDH EpiTracks® system and included clinical cases confirmed by the MSDH Public Health Laboratory and/or the Centers for Disease Control, private reference laboratories, and blood banks. Questions were asked about environmental conditions around patients' homes, their neighboring properties, as well as various behaviors such as mosquito source reduction, time spent outdoors, and repellent use. A self-addressed, stamped envelope was included with each survey for its return.

Forty-three of the 400 questionnaires were returned by post office with no forwarding address, so we used Internet people searches in an attempt to find current addresses for them (and they were re-sent). Twenty-three of the re-sent questionnaires were returned, leaving us to *assume* that at least 377 patients received the survey.

4.3.2 Demographic and socio-economic data

Socio-economic and educational level data were obtained from the U.S. Census Bureau for each tract or area where case(s) occurred (Figure C.4), using the date range of 2008 through 2013 using a previously published methodology (Rios et al. 2006, United States Census Bureau 2013). No attempt was made to obtain these data for each individual patient as that would entail stricter IRB review. Due to budgetary and personnel reasons, no negative control group was established since many people become

infected with WNV but remain asymptomatic, i.e., we would never know who was truly negative.

4.4 Statistical analysis

Staff statisticians in both our agencies were consulted and the consensus was that (only) descriptive statistics are applicable to these data since there was no true negative control group. These results are intended to serve as the basis for future hypothesis testing research by the Mississippi Department of Health which will include appropriate negative control groups.

4.5 Results and Discussion

4.5.1 Demographic and socio-economic results

A total of 134 (36%) questionnaires were completed and returned which geographically mirrored the distribution of most human WNV cases occurring 2008-2013 (Figure 4.1). Analysis of patient information obtained in the questionnaires is presented and discussed below.

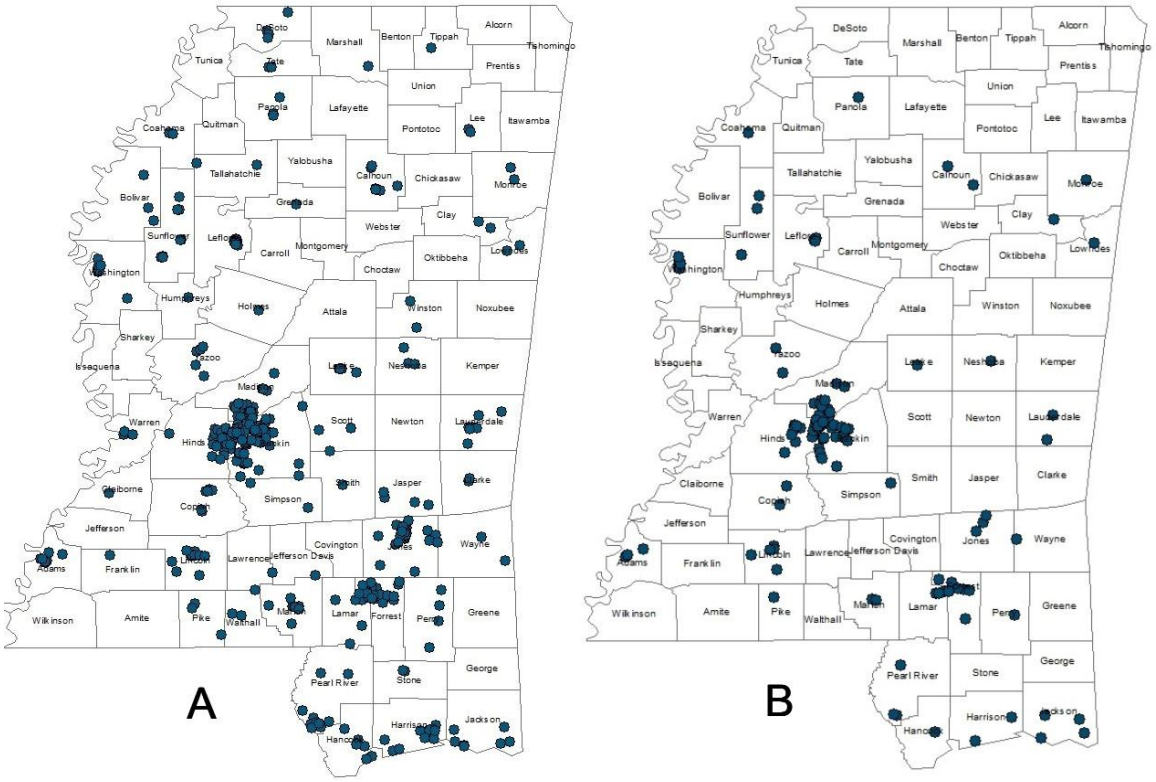

Figure 4.1 Map A – Location of all human WNV occurring in MS 2008-2013. Map B – Location of survey respondents.

Incidence of WNV infection during the time period 2008-2013 by race is provided in Table 4.1. Interestingly, the highest incidence rate occurred in whites (17.6/100,000). This is in contrast to studies in Mississippi with St. Louis encephalitis (SLE) which showed that the incidence was higher in black populations (Monath 1980). Additionally, the incidence rate of all WNV cases was higher in males (18.7/100,000) compared to females (13.1/100,000), which might be due to more outdoor chores/activities in males. Other studies have shown that this disparity is even more pronounced when analyzed by neuroinvasive disease cases only (true also for our cases) (Murray et al. 2013). The socio-demographic profile of those who responded is as

follows: the mean age was 58.7 years (range 19-88 years) and 47.8% (52.2%) of the respondents were female (male). Racial makeup of WNV patients included 79% white, 11% black, 1% Asian, and 9% undetermined (information absent from MSDH records). Those diagnosed with WNV fever represented 59% of respondents; the remaining 41% had the more serious neuroinvasive disease. When analyzed by census data for each tract, the average median income of *all* WNV cases in Mississippi during the time period was $44,296.82 (Table 4.1), and the average percentage of patients with at least a high school diploma or higher was 83%. In contrast to one study in Texas (Rios et al. 2006), our WNV patients had a median income level *above* that of the general population, i.e., they were not from a lower socioeconomic class (Table 4.1).

Table 4.1 Average median income of Mississippi WNV cases by race, 2008-2013, based on census tracts with human cases.

Race	Avg. Median Income	% Below Poverty Level
White	47,645	5.7%
Black	32,110	20.0%
Hispanic	42,576	16.6%
Asian	98,175	0%
Unknown	46,734	10%
Overall	**$44,296**	**9.2%**
Statewide	**$39,031**	**24.0%**

4.5.2 Survey Results

The raw data for all the responses below are provided in Appendix C.5

4.5.2.1 Property descriptions and environmental conditions

Approximately 2/3's of Mississippi WNV patients in this study reported living within a town/city limit. Accordingly, one might expect some level of public works or

sanitation in their areas, even mosquito control. In fact, 103/134 (76%) of respondents reported a mosquito spray truck in their city or county. This, of course, does not mean these spray trucks are being actively utilized. A previous survey of mosquito control practices in Mississippi found that almost half of spray programs were located in towns with fewer than 3,000 people and program managers reported limited financial resources for mosquito control (Edwards et al. 2009). Ninety-three percent (124/134) of respondents reported living in single family dwellings and 100% reported having air conditioning. Seventy four percent (99/134) reported having an open deck or unscreened porch on their house, and 104/134 (78%) reported intact screens on their windows. Most of our respondents 102/134 (76%) reported wooded areas near their houses, 119/134 (89%) said they have a yard or garden, and about a third (31%) had a storm drain nearby. Ninety percent (120/134) reported no standing water under their houses (and another 9% of them said they did not know). When asked about wildlife around their homes, 63/134 (47%) said they have a bird feeding station near their house and 57/134 (43%) said they have bird houses located on their property.

4.5.2.2 Personal protection behaviors and homeowner mosquito control activities.[2]

Approximately 80% (109/134) of responders reported removing trash and debris from their property and dumping containers holding water, while 72/134 (53%) reported cleaning out their gutters. As for sewage disposal, 85/134 (63%) reported being on a city sewer and 49/134 (37%) had either a septic tank or treatment plant in their yards.

[2] Not all responses are provided here. See Appendix Figures C.3 and C.5 for additional information.

However, only 26/134 (19%) of respondents said they properly serviced and maintained their on-site sewage systems. This is important because the primary vector of WNV in Mississippi, *Culex pipiens quinquefasciatus*, is known to breed in effluent from failing or poorly functioning on-site wastewater systems, (Barrera et al. 2008, Mackay et al. 2009, Pires and Gleiser 2010).

Interestingly, respondents reported extensive outdoor activity which may have led to increased mosquito exposure. Eighty-nine percent (119/134) of our WNV patients reported sitting outside on a porch or patio, and 79/134 (59%) said they spent 5 days or more per week outdoors. This may be due to patients being retired or otherwise not working (Gibney et al. 2012). Of the people who said they spent time outdoors, 121/134 (90%) reported spending at least 2 hours outside. Over a third of the respondents (35%) reported being outside more than 4 hours each time. This compares with other studies including a survey of over 800 Mississippi residents which found 48% of respondents spent >30 minutes outside more than 4 days a week (Slavinski and Jones 2004). Only about half (71/134) of the people reported avoiding mosquitoes when they were most active, and for personal protection when outdoors, 112/134 (83%) said they used repellents; 51/134 (38%) said they used outdoor fogs and sprays; 41/134 (30%) said they wore long-sleeved shirts or long pants; and 22/134 (16%) reported repairing window screens.

Use of protective behaviors among respondents in this survey roughly matched a wider, more comprehensive MSDH survey of mosquito control among Mississippi residents (but not known to be WNV patients) (Table 4.2). One exception was repairing screen-wire windows and doors. The 2003 survey reported 56% repaired screens, but our

survey showed only 16%. Whether or not this impacted the risk of WNV infection is unknown. Interestingly, in our study, *after* having WNV infection, 36/126 (29%) of responders said that their personal protective behaviors did not change.

When asked where WNV patients obtained their information about mosquito control and WNV, 98/134 (73%) reported television, 65/134 (48%) reported newspaper, 55/134 (41%) internet, 19/134 (14%) printed materials such as brochures, and 18/134 (13%) from the radio. These results are enlightening because considerable health department resources have been devoted in the past to print materials and radio spots warning residents about WNV (Liz Sharlot, Mississippi Department of Health, personal communication). Our study suggests that television would be the best medium for such announcements, a finding consistent with other studies (McCarthy et al. 2001, Averett et al. 2005, LaBeaud et al. 2007).

Table 4.2 Use of personal protection measures against mosquitoes in Mississippi.

Protection Method	2003 Survey[1]	Current Survey
Avoid activity when mosquitoes active	47%	53%
Wear long-sleeved shirts and pants	57%	31%
Using repellents	67%	84%
Screening of windows and doors	56%	16%
Clean out gutters	56%	54%
Remove standing water	63%	81%

[1]Slavinski 2004.

Environmentally, a majority of respondents said they paid attention to mosquito breeding sites around their home and made efforts to eliminate them. Other studies have found similar results (Averett et al. 2005, Wilson et al. 2005). This fact may demonstrate that clean-up campaigns and other educational efforts by the health department have been successful. Another possible environmental factor contributing to WNV ecology identified in this study was the presence of bird feeding, nesting, and attracting devices near homes (40-47%). These may have brought high numbers of bird reservoirs of WNV into close proximity of patients.

4.6 Conclusions

This survey highlights several important epidemiological and behavioral aspects of WNV patients. For one thing, Mississippians in WNV endemic areas do not get their information about WNV from brochures, other print materials, or radio spots. Television and internet were the primary avenues for patient education. The amount of time spent outdoors seemed associated with WNV infection which is consistent with previous studies (McCarthy et al. 2001). Only one of 134 respondents in this study spent less than 2 hours outdoors one day a week. A more controlled study of outdoor activity by people living in WNV endemic areas is warranted. Interestingly, the use of personal protection techniques such as repellents did not usually change from before WNV infection to after. From discussion with WNV patients (not part of this survey), we have found them to be unconcerned about repeat infection, believing that they now have life-long immunity. However, these patients fail to realize that there are several other mosquito-borne diseases present in Mississippi. This too, highlights the need for continued targeted educational efforts.

This study has several limitations. We depended on self-reporting to measure the frequency of environmental conditions (such as breeding sites) and personal protective behaviors against WNV. There might be a variety of reasons why some patients failed to respond, including inability to read. We made no additional efforts to re-survey these non-responders, so it is unknown if there are significant differences in responders and non-responders in risk factors, personal protection behaviors, etc. Further, due to lack of resources, we made no effort to try to validate the responses of each participant. Frequency data might be overestimated due to participant attempts to please interviewers with their answers. Despite these limitations, our study will help public health personnel achieve their goals to promote health and educate the public about personal protective behaviors for mosquito-borne diseases, and thus, reduce risk of future infections. Our work will also lay the groundwork for future studies such as widespread sero-surveys of populations to accurately assess WNV infection rates and visual household surveys (onsite environmental surveys) to validate responses. Also, hypothesis-driven studies of specific risk factors associated with WNV infection are in order and currently planned.

4.7 Acknowledgements

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APPENDIX A

SUPPLEMENTAL MATERIAL FOR CHAPTER II

Figure A.1 Setting CO_2 baited CDC light trap in location 1 in the Pearl River Wildlife Management Area.

Figure A.2 Setting CO_2 baited CDC light trap in location 2 in the Pearl River Wildlife Management Area.

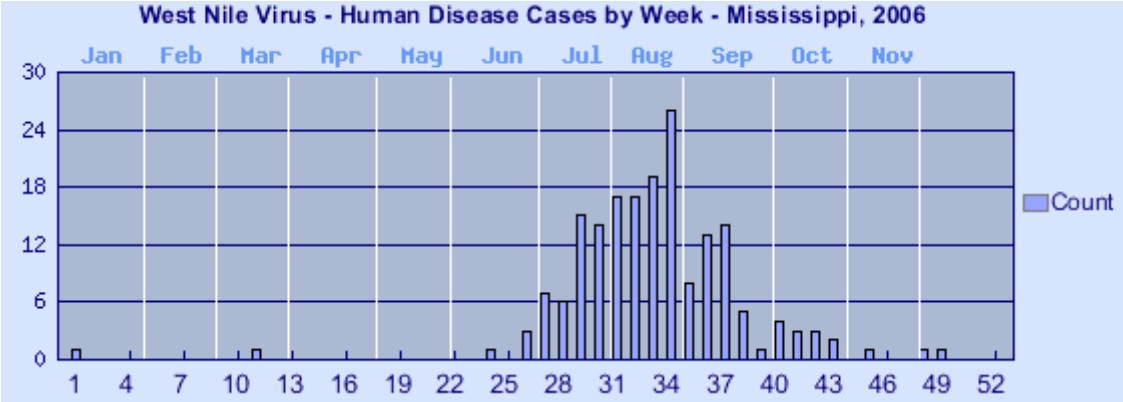


Figure A.3 Typical human WNV season in Mississippi.

Source: U.S. Geological Survey.

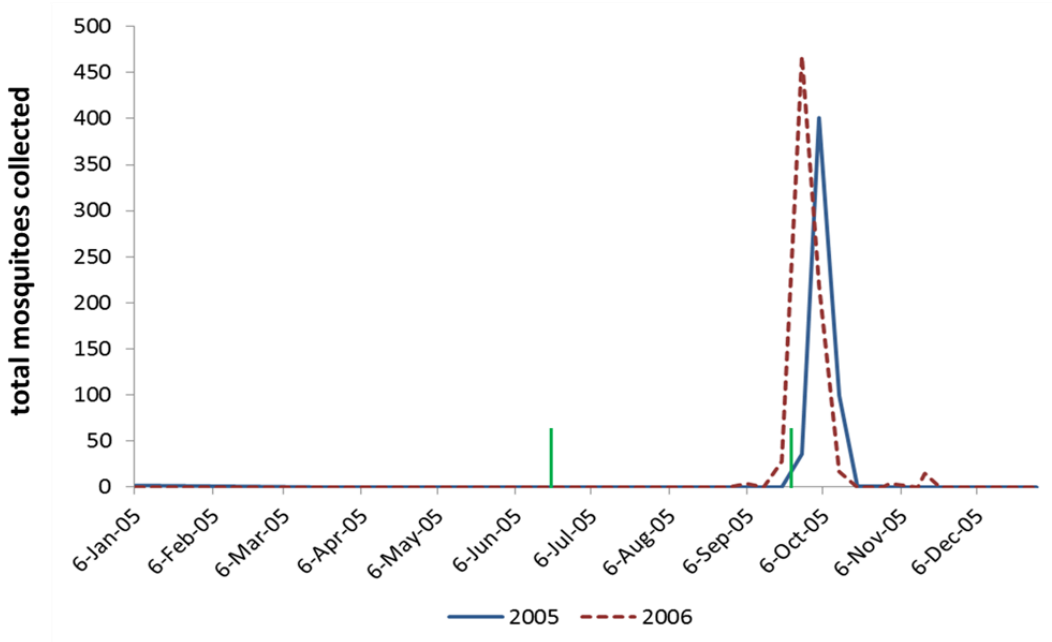


Figure A.4 Seasonality and abundance of *Culex nigripalpus.*

The days between mid-June and mid-September (green lines) represent typical West Nile season in Mississippi.

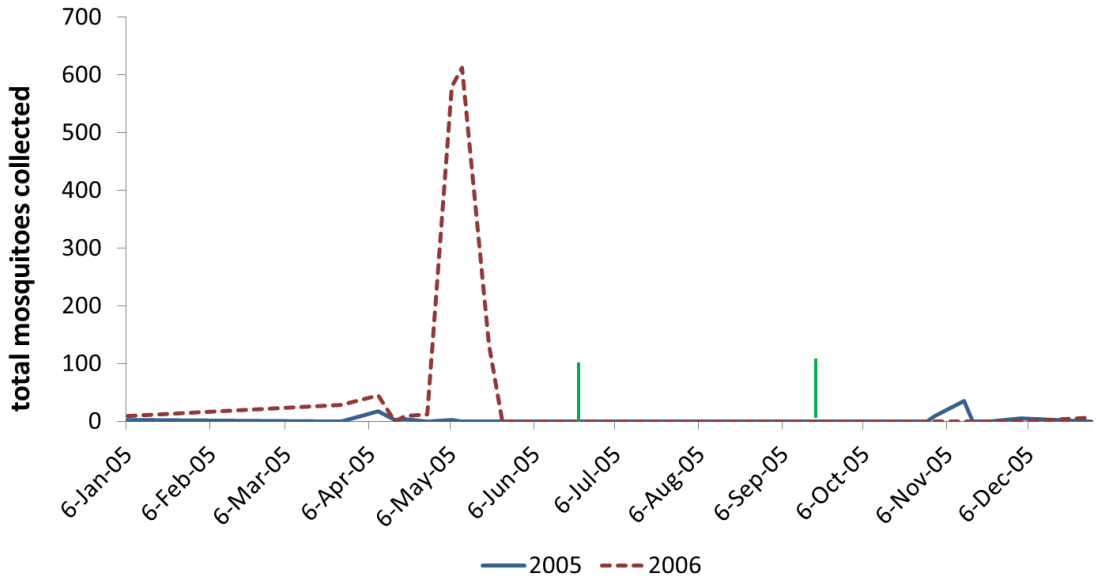


Figure A.5 Seasonality and abundance of *Culex restuans.*

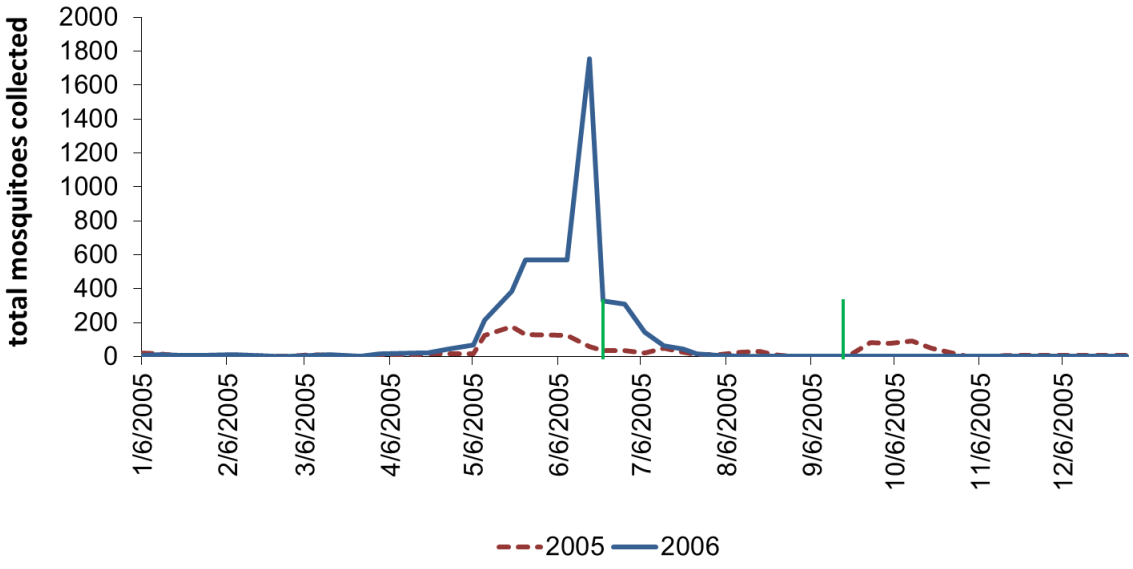


Figure A.6 Seasonality and abundance of *Culex salinarius.*

APPENDIX B

SUPPLEMENTAL MATERIAL FOR CHAPTER III

Figure B.1 Gravid trap set-up at a Jackson Metro sampling site.

All gravid traps were baited with a mixture of fish emulsion and water.

VectorTest®

West Nile Virus / Saint Louis Encephalitis Antigen Panel Assay

VecTOR Test Systems, Inc.

Intended Use

The VectorTest® West Nile Virus (WNV) / Saint Louis Encephalitis (SLE) Antigen Panel Assay is a rapid immunochromatographic assay intended for the qualitative determination of WNV and SLE antigens in infected mosquitoes. Results from this assay can enable public health teams to:

- Continuously monitor mosquito vectors
- Focus vector control and eradication efforts
- Deliver cost-effective prevention of disease

Summary

Assays which detect the disease-causing agents and pathogens in field populations of arthropods, such as mosquitoes, make it possible to monitor the spread of the disease, to identify areas where there is risk of contracting disease, and to more efficiently target arthropod control measures. By monitoring infection rates and viral activity in nature, it may be possible to predict the threat of epidemic transmission in a population. While growth of the virus in cell culture or PCR-based molecular methods remain the standard for virus identification, the availability of a rapid, stable, simple, sensitive and specific diagnostic tool makes virus surveillance more expedient and cost-effective.

Monoclonal antibodies against SLE, WNV, and the Flavivirus group have been employed in developing enzyme-linked immunosorbent assays (ELISA) that have been valuable tools in epidemiological studies and in assessing the risk and identification of vectors. Specific antibodies, extensively characterized by ELISA, have been employed in the development of the VectorTest® WNV/SLE Antigen Panel Assay. The use of the ELISA in monitoring infection rates in vector mosquitoes has been a great improvement over the methods of virus isolation in cell culture or in mice. However, the ELISA format is not always practical nor expedient enough to give the information required in medical threat assessments. The

ELISA is a multi-component, 4-6 hour assay requiring specialized equipment, refrigeration of reagents, and highly trained personnel. Access to such facilities and equipment is usually unavailable in the field where the specimens are obtained and testing must wait until it can be done in a suitable laboratory environment.

The VectorTest® WNV/SLE Antigen Panel Assay is a rapid wicking assay that identifies the presence or absence of viral antigen specific to WNV or SLE in mosquitoes. The assay is a rapid, one step procedure using a wicking test strip. Rapid results, ambient storage and lack of specialized equipment needed in testing samples are the big advantages of the wicking WNV/SLE/WEE antigen assay over the ELISA, and prior training is not necessary.

This type of immuno-chromatographic WNV/SLE assay detected WN viral antigen in all of the pools containing a minimum of 3.5 – 4.0 $\log^{10}$ PFU/ml of virus (estimated Vero cell plaque assay equivalent). No evidence was found of cross reaction or false positives in any of the tests (Nasci et al, 2003). Dilutions of each virus showed that virus titers of 3.8 and 3.4 $\log_{10}$ PFU/ml for WN and SLE viruses can be detected with this method (Ryan et al 2002).

Principle

The VectorTest® WNV/SLE Antigen Panel Assay is based on the dual monoclonal antibody "sandwich" principle. The test is initiated by placing one VectorTest™ WNV/SLE dipstick into a 250 µl (0.25 ml) of ground mosquito extract. Antigen present in the solution binds to the specific antibody with a gold sol particle label. As the antigen-antibody-gold complexes migrate through the test zone containing immobilized WNV, and SLE antibodies, they bind to the corresponding immobilized antibodies forming a "sandwich". The unbound dye complexes migrate out of the test zone and can be captured later in the control zone. A reddish-purple line develops on the specific area of the test zone when antigen is present. The control line in the control zone, farthest from the sample, should always develop provided the test has been carried out correctly.

Reagents

VectorTest® WNV/SLE Antigen Panel Assay is available as a unit of 50 single-use dipsticks. Each Vector Test™ WNV/SLE Antigen Panel Assay kit contains the following:

- 50 VectorTest™ WNV/SLE Antigen Panel Assay dipsticks in canisters with desiccant cap
  - **Test Zone:** Monoclonal antibodies to WNV and SLE immobilized on a membrane
  - **Control Zone:** Polyclonal goat antibody to mouse immunoglobulins immobilized on a membrane
  - **Conjugate Pad:** Gold complexed to monoclonal antibodies to flavivirus
- Grinding Solution (2 x 44 mL)

Storage and Stability

The VectorTest® WNV/SLE Antigen Assay dipsticks and unopened Grinding Solution are stable up to the expiration date when stored at room temperature (10-30°C or 50-86°F). To obtain good test results, dipsticks should be kept tightly closed in the provided container until ready for use.

Specimen Collection and Preparation

Mosquitoes: Use mosquitoes captured using the method(s) which best support(s) the sampling of encephalitis or West Nile fever vectors in the geographic area in which testing is to be done.

Storage: Mosquitoes should be used immediately or should be dried for later use. Homogenized samples of mosquitoes should be stored at -20°C until they can be processed.

Procedure

Materials Provided

- VectorTest® WNV/SLE Antigen Panel Assay dipsticks (total of 50) in two canisters with desiccant cap
- 2 bottles of Grinding Solution (44 mL each)
- 50 culture tubes
- 200 copper coated BBs in a container
- 50 conical tubes
- 2 blue pestle homogenizers
- 5 ten-hole tube racks
- Instructional insert

Materials Required (but not provided)

- Vortex machine
- Pipette
- Timing device
- Centrifuge (optional)
- Motorized grinder (optional)

Guidelines for Handling the Grinding Solution

Before use, the Grinding Solution should be mixed by gently inverting the bottle five times. Use clean pipet tips for removing solution from bottle.

Procedure Outline

1. Place female mosquitoes into a plastic culture tube provided in the kit.

2. Dispense 1 mL of Grinding Solution onto the mosquitoes and add four copper-coated BBs provided in the kit. Snap-cap the tube firmly.

3. Vortex the capped tube for 1-2 minute at high speed until the mosquito pool is homogenized into a slurry. (Allow debris to settle down briefly, cut the tip of the pipet tip to increase the orifice reasonably and pipet out 0.250 ml suspension into assay tube as shown in next step.)

4. Dispense 250 µl of mosquito homogenate into a conical tube provided, place the tube into the tube stand provided, and insert a test strip from the canister with the arrows pointing down. (Replace the desiccant cap on the canister to protect the remaining strips from moisture.)

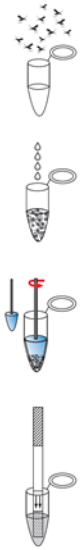
Figure B.2 VectorTest® package insert describing testing procedure.

Wait 15 minutes for the test to be completed.

5. Determine the test results by removing the test strip and comparing it to the pictorial sample provided on the back of this insert.

Maceration of smaller pools of mosquitoes can be performed with blue grinders and eppendorf tubes provided. Maceration of larger mosquito pools can be performed by vortexing with BBs in capped- culture tubes provided in the kit. Depending on the type and size of mosquitoes, 1 to 25 mosquitoes can be macerated in 250µL of GS in eppendorf tube and upto 50 in 1 - 2.5 ml GS in culture tubes. Note that excessively thick suspension will not allow proper wicking of the sample through the dipstick and can affect the performance of the assay.

Alternate Procedure Outline

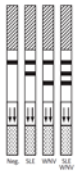


1. Place female mosquitoes into the conical grinding tube provided in the kit. Place the tube into the tube stand provided.
2. Dispense 250µL of the Grinding Solution from a GS bottle onto the mosquitoes.
3. Place the pestle provided in the kit into the grinding tube and vigorously rotate it to homogenize the mosquitoes. The pestle can be reused if washed between uses. A mechanical battery operated pestle homogenizer can be used for this – however, limit the amount of foam produced.
4. Place a test strip from the canister into the mosquito suspension in the grinding tube with the arrows pointing down.

Replace the desiccant cap on the canister to protect the remaining strips from moisture.

Wait for the test to be completed. Results can be read ~20-30 minutes or later.

5. Determine the test results by removing the test strip and comparing it to the pictorial sample provided.



Quality Control Results

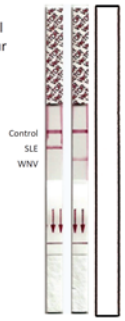
The test results are not valid if the control line does not develop. These results should be disregarded, the dipstick discarded, and the test run again. The test should only be interpreted as positive if two or more lines develop, one of these lines being the "Control" line.

Results

The presence of only a control line on the dipstick indicates a negative test result. The presence of two or more lines indicates the presence of WNV and/or SLE antigen. Results can be read 15-30 minutes after performing the assay.

Test Strip Comparison

Place your strip in the box to the right. Align the control line (generally darker) of your strip with the control line of the sample strip below and compare signal pattern(s).



Limitations

The testing volume of the homogenized solution should be 250 µl +/- 25 µl. The amount of mosquito debris in larger quantities may interfere with the interpretation of results. The amount of foam remaining in the solution after homogenization should be minimized.

Specific Performance Characteristics

This immuno-chromatographic method in the WNV/SLE panel assay detected WN viral antigen in all of the pools containing a minimum of 3.5 – 4.0 $\log^{10}$ PFU/ml of virus (estimated Vero cell plaque assay equivalent). No evidence was found of cross reaction or false positives in any of the tests. Dilutions of each virus showed that virus titers of 3.8 and 3.4 $\log_{10}$PFU/ml for WN and SLE viruses can be detected with this method (Ryan et al 2002). One infected mosquito could be detected in a pool of 50 mosquitoes ground in a 2.5 ml sample (Nasci et al 2003, CDC Fort Collins, CO.

Effect of Grinding Solution on Viral Propagation

The Grinding Solution provided with the VectorTest® kit contains detergents and preservatives and affects the propagation of viruses. Precautionary handling steps are always recommended.

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Insert was revised 9/2012.

VecTOR Test Systems, Inc.
3537 Old Conejo Road, Unit 101
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Fax: 805-376-8480
www.vectortest.com
email: admin@vectortest.com

Figure B.2 (continued)

2013	Total No. of collections (average number of mosquitoes per collection)	Collection Dates	Number of positive collections	MIR	Human onset of symptoms	Positive humans within 4 miles of trap	Miles between WNV positve human and closest trap	Days between positive trap and 1st human onset
Total								
Greenville	86 (136)	6/3 thru 9/5	11	0.94				
No human cases					*****	*****	*****	*****
Greenwood	22 (132)	6/19 thru 9/4	9	3.1				
Human 1					7/15/2013	6/19/2013	2.05	26 days
Human 2					7/16/2013	6/19/2013	1.23	25 days
Golden Triangle Area	46 (91.5)	5/31 thru 8/3	4	0.95				
No human cases					*****	*****	*****	*****
Jackson Metro	34 (20.75)	5/30 thru 9/3	3	4.38				
Human 1(Jackson)	"	"	"	"	7/26/2013	none +	3.1	none
Human 2 (Jackson)	"	"	"	"	8/2/2013	7/17/2013	2.8	16 days
Human 3 (Jackson)	"	"	"	"	8/10/2013	7/17/2013	1.8	24 days
Human 4 (Jackson)	"	"	"	"	8/13/2013	7/17/2013	2.5	27 days
Human 5 (Jackson)	"	"	"	"	8/13/2013	7/17/2013	2.01	27 days
Human 6 (Jackson)	"	"	"	"	8/25/2013	7/17/2013	3.5	39 days
Human 7 (Jackson)	"	"	"	"	8/27/2013	none +	3.6	none
Human 8 (Jackson)	"	"	"	"	9/1/2013	7/17/2013	1.8	46 days
Brookhaven	2 (14)	7/24	0	0				
Human 1					6/18/2013	none +	3.5	too little data
McComb	2	7/24	1	100*				
No human cases					*****	*****	*****	too little data
Hattiesburg (Forrest)	29 (104.5)	6/26 thru 7/25	11	3.63				
Human 1	"	"	"	"	7/29/2013	7/9/2013	1	20 days
Human 2	"	"	"	"	8/11/2013	7/9/2013	0.66	31 days
Human 3	"	"	"	"	8/20/2013	7/9/2013	7	
Human 4	"	"	"	"	9/1/2013	7/9/2013	1.2	42 days
Human 5	"	"	"	"	9/11/2013	7/9/2013	0.5	53 days
Laurel	12	6/27 thru 7/22	3	5.65				
Human 1	"	"	"	"	8/5/2013	7/3/2013	9	33 days
Human 2	"	"	"	"	8/8/2013	7/3/2013 (7/10)	0.7 (.004)	36 days (29 days)
Biloxi/Gulfport	74	5/21 thru 8/7	0	0				
No human cases					*****	*****	*****	*****

Figure B.3 Mosquito, VectorTest®, and human WNV case data for selected study sites in Mississippi, 2013.

2014	Total No. of collections (average number of mosquitoes per collection)	Collection Dates	Number of positive collections	MIR	Human onset of symptoms	Positive humans within 4 miles of trap	Miles between WNV positve human and closest trap	Days between positive trap and 1st human onset
Total								
Greenville	32 (74.2)	7/16 thru 8/19	17	7.16				
Human 1					8/2/2014	7/21/14	2.65	12 days
Greenwood	no trapping							
No human cases					*****	*****	*****	*****
Golden Triangle	22 (40)	6/4 thru 7/29	0	0				
Human 1 (Columbus)	3 (17.3)	6/4 thru 7/29	"	"	9/8/2014	none +	2.4	didn't catch +
Human 2 (West Point)	5 (26)	6/4 thru 7/29	"	"	10/24/2014	none +	1.8	didn't catch +
Jackson Metro	89 (83)	6/19 thru 8/20	25	3.38				
Human 1 (Brandon)	9 (69)	6/19 thru 8/20	1	1.61	7/3/2014	8/13/2014	0.5	41 days after
Human 2 (Brandon)	"	"	"	"	7/19/2014	8/13/2014	0.6	41 days after
Human 3 (Brandon)	"	"	"	"	8/3/2014	none +	1.6	0
Human 4 (Brandon)	"	"	"	"	9/14/2014	none +	0.7	0
Human 5 (Jackson)	65 (62.4)	6/10 thru 9/9	16	3.94	7/26/2014	7/15/2014	2.4	11 days
Human 6 (Jackson)	"	"	"	"	8/13/2014	7/15/2014	0.6	29 days
Human 7 (Jackson)	"	"	"	"	8/14/2014	7/15/2014	0.8	30 days
Human 8 (Jackson)	"	"	"	"	8/21/2014	7/15/2014	1.89	37 days
Human 9 (Jackson)	"	"	"	"	8/22/2014	7/15/2014	1.7	38 days
Human 10 (Jackson)	"	"	"	"	8/24/2014	7/15/2014	1.48	40 days
Human 11 (Jackson)	"	"	"	"	8/25/2014	7/15/2014	0.6	41 days
Human 12 (Jackson)	"	"	"	"	9/2/2014	none +	2.4	0
Human 13 (Jackson)	"	"	"	"	9/6/2014	7/15/2014	1.57	52 days
Brookhaven	20 (63.2)	6/16 thru 9/2	1	0.79				
No human cases					*****	*****	*****	*****
McComb	18 (39.5)	6/16 thru 9/2	2	2.81				
No human cases					*****	*****	*****	*****
Hattiesburg (Forrest)	25 (64.7)	6/16 thru 9/2	16	9.9				
Human 1	"	"	"	"	8/15/2014	7/2/2014	1.05	37 days
Human 2	"	"	"	"	8/22/2014	7/2/2014	0.7	44 days
Human 3	"	"	"	"	8/27/2014	7/2/2014	4.1	
Laurel	25	6/15 thru 9/2	5	2.57				
Human 1					8/22/2014	7/31/2014	0.4 (1.2)	22 days
Biloxi/Gulfport	16 (15.4)	6/9 thru 8/19	0	0				
Human 1 (Gulfport)	7 (12.1)	6/9 thru 8/19	"	"	8/18/2014	none +	2.5	didn't catch +

Figure B.4 Mosquito, VectorTest®, and human WNV case data for selected study sites in Mississippi, 2014.

2013 Sample #	Real Time RT-PCR Result	Vectest Results	2014 Sample #	Real Time RT-PCR Result	VectorTest Results
340	pos	pos	172	neg	Pres Pos
427	pos	pos	221	pos	pos
431	pos	pos	292	pos	pos
441	neg	pres pos	315	equ	pos
469	pos	pos	326	pos	pos
475	pos	pos	346	equ	pos
476	pos	pos	347	equ	pos
478	pos	pos	348	pos	pos
480	pos	pos	349	pos	pos
482	pos	pos	359	pos	pos
487	pos	pos	360	pos	pos
488	pos	pos	366	pos	pos
506	pos	pos	423	pos	pos
515	pos	pos	424	pos	pos
594	neg	pres pos	440	pos	pos
596	pos	pos	451	pos	pos
673	pos	pos	452	pos	pos
678	pos	pos	453	pos	pos
681	pos	pos	455	pos	pos
682	pos	pos	458	pos	pos
683	pos	pos	460	pos	pos
687	pos	pos	461	pos	pos
693	pos	pos	468	pos	pos
698	pos	pos	492	pos	pos
707	pos	pos	494	pos	pos
714	pos	pos	496	pos	pos
718	pos	pos	510	equ	pos
721	pos	pos	515	pos	pos
723	pos	pos	566	pos	pos
728	pos	pos	576	pos	pos
731	pos	pos	618	pos	pos
746	pos	pos	624	pos	pos
748	pos	pos	629	pos	pos
787	pos	pos	630	pos	pos
802	pos	pos	644	pos	pos
813	pos	pos	682	pos	pos
830	pos	pos	691	pos	pos
919	pos	pos	692	neg	pres pos
992	pos	pos	695	pos	pos
993	pos	pos	696	equ	pos
997	pos	pos	698	pos	pos
1006	pos	pos	719	pos	pres pos
1019	pos	pos	721	equ	pos
			722	pos	pres pos
			723	pos	pos
			724	pos	pos
			735	pos	pres pos
			763	pos	pos
			770	pos	pos

Figure B.5 Real-time RT-PCR confirmation of 2013-2014 VectorTest®+ mosquito pools.

Testing conducted by the Centers for Disease Control and Prevention.

APPENDIX C

SUPPLEMENTAL MATERIAL FOR CHAPTER IV

MISSISSIPPI STATE DEPARTMENT OF HEALTH

February 4, 2014

Wendy Varnado
Principal Investigator
MSDH
PO Box 1700
Jackson, MS 39216

Re: *The Ecology and Epidemiology of West Nile Virus in MS*
MSDH IRB Protocol #011114

Dear Ms. Varnado:

Your research application for the above referenced project was approved by expedited review of the MSDH IRB chairperson as authorized by 45 CFR 46.110. Please refer to protocol **#011114** on all documents or correspondence with the IRB concerning this research. You are also required to inform the IRB of any modifications or changes to your protocol immediately.

Please be informed that the IRB must review the status of the research at least annually until the research is finished. Continued approval of this study is contingent on informing this office of any changes in the research protocol. If the study continues beyond one year from the IRB approval date, you must request a continuation. It is suggested that you submit a continuation to the IRB at least 60 days in advance of end of the approval period on February 4, 2015.

Please refer to Section 4.0 of the MSDH general agency manual for information related to IRB submission and review.

Sincerely,

Meg Pearson, PharmD, MS
Chair, Institutional Review Board

570 East Woodrow Wilson • Post Office Box 1700 • Jackson, MS 39215-1700
1-866-HLTHY4U • www.HealthyMS.com

Figure C.1 Mississippi State Department of Health IRB approval letter and protocol number.

West Nile Survivor Questionnaire

Name: _____ **County:** _____

Address: _____

1. Do you live within the town/city limits? ☐ Yes ☐ No ☐ Don't Know
2. Type of housing structure ☐ Single family ☐ Duplex/triplex ☐ Apt. complex ☐ Other
3. Is air-conditioning used in your home? ☐ Yes ☐ No
4. If yes, what type of air-conditioning is it? ☐ Central ☐ Window/Wall ☐ Don't Know
5. Is there an open deck or unscreened porch for your home? ☐ Yes ☐ No ☐ Don't Know
6. Are there intact screens on **all** of the windows than can be opened in your home? ☐ Yes ☐ No ☐ Don't Know
7. To get rid of wastewater or sewage from your household, is your home connected to: ☐ Municipal sewage system ☐ Individual on-site treatment plant ☐ Septic tank ☐ Other (specifiy:_____)
8. Are there any wooded lots or parks within a block of this residence? ☐ Yes ☐ No
9. How many days per week would you estimate that you spend outdoors at your home? ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7
10. During the summer months, do you frequently spend time outdoors at your home, such as in the garden, on a porch or patio? ☐ Yes ☐ No
11. How many hours per day would you estimate that you spend outdoors in a garden, on a porch or patio during the warmer months of the year? ☐ less than 1 ☐ 2 ☐ 3 ☐ 4 ☐ more than 4
12. Do you know if your city or town has a mosquito spray truck? ☐ Yes ☐ No ☐ Don't Know
13. Where do you get information about mosquito control and West Nile virus? ***Check all that apply*** ☐ Television ☐ Newspaper ☐ Internet ☐ Radio ☐ Brochures/flyers
14. What mosquito control prevention activities around the house do you perform ***Check all that apply*** ☐ Dump containers ☐ Clean out gutters ☐ Remove trash, garbage, tires & other debris ☐ Fix and/or correctly maintain septic tanks/sewer systems
15. What **personal** mosquito protection techniques do you use? ***Check all that apply*** ☐ Avoid mosquitoes when they're most active ☐ Long sleeve shirt/pants ☐ Mosquito repellents ☐ Repair window screens ☐ Outdoor fogs/sprays

Continued on other side

Figure C.2 Survey sent to WNV survivors asking questions pertaining to environment immediately surrounding the home they lived in when they acquired WNV.

16. Did your use of personal protective techniques change or increase after you contracted West Nile Virus? ☐ Yes, tremendously ☐ Yes, some ☐ No, not much ☐ No, not at all

Environmental Survey	Your Property	Neighboring Properties
House on pillars (not on concrete slab)	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Water standing under house	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Low lying areas around home/puddles	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Outdoor yard/garden area	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Abandoned lot	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Wooded lot/field	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Gutters on home	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Corrugated pipe at base of gutter downspout	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
French drain	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Bird feeding stations	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Bird nesting boxes/bird houses	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Birdbath	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Bushes/shrubs	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Trees	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Overgrown lawn	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Storm drain/storm sewer	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Lake or large pond	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Stream or open ditch	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Unmaintained swimming pool	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Uncovered boat, canoe, kayak	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Standing water under home from rain/runoff	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Leaky plumbing under home	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Rain barrels	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Potted plants	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Kiddy pool	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Kids toys or lawn ornaments	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Pet dishes	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Ornamental pond without fish	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Watering cans/buckets	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Old tires	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know
Any item covered with a tarp	☐ Yes ☐ No ☐ Don't Know	☐ Yes ☐ No ☐ Don't Know

Figure C.2 (continued)

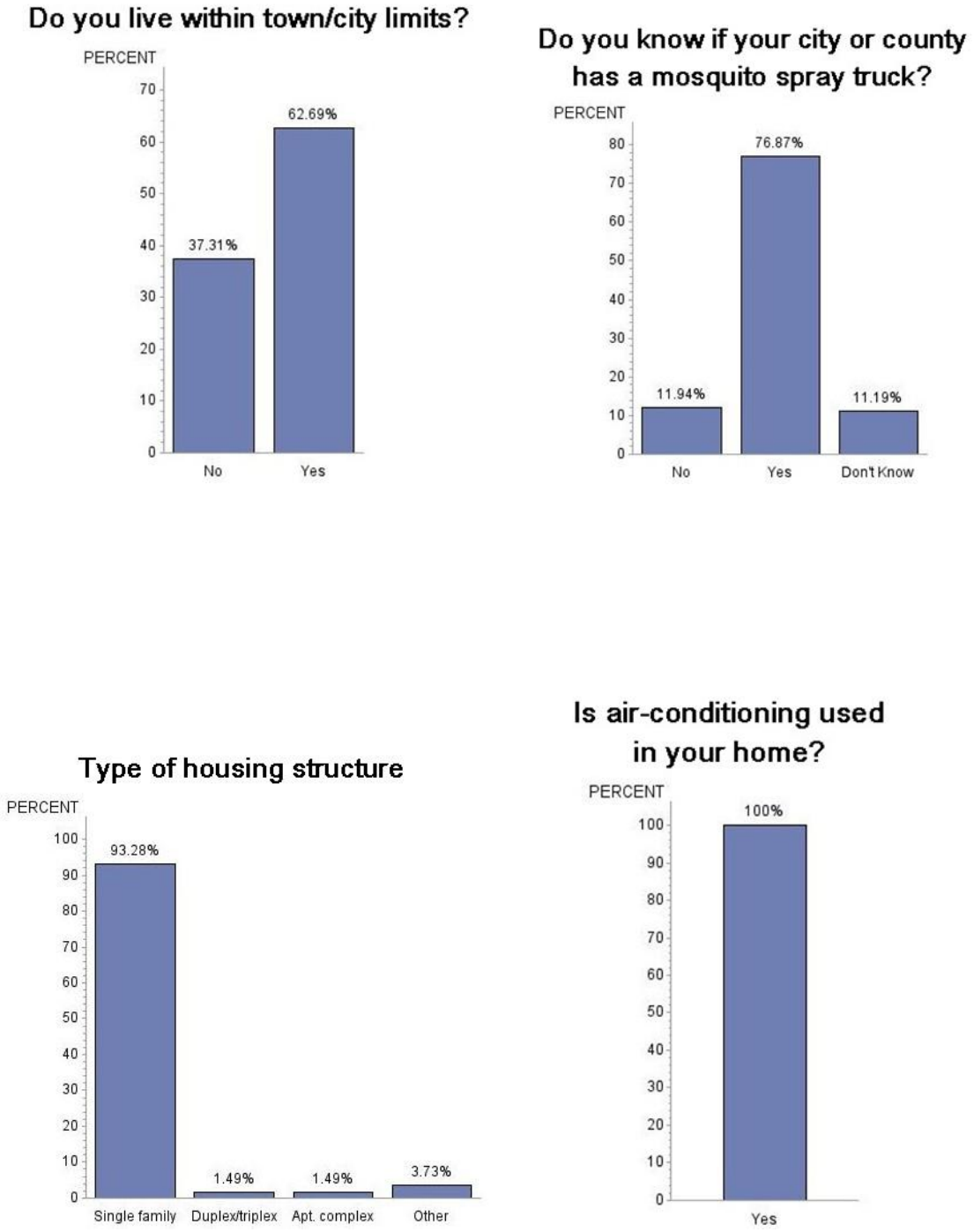
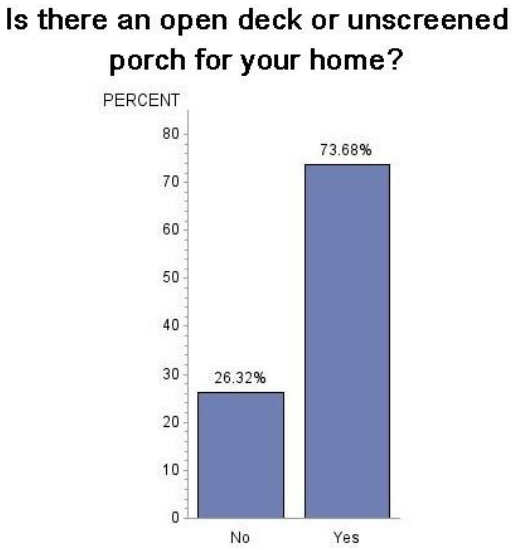
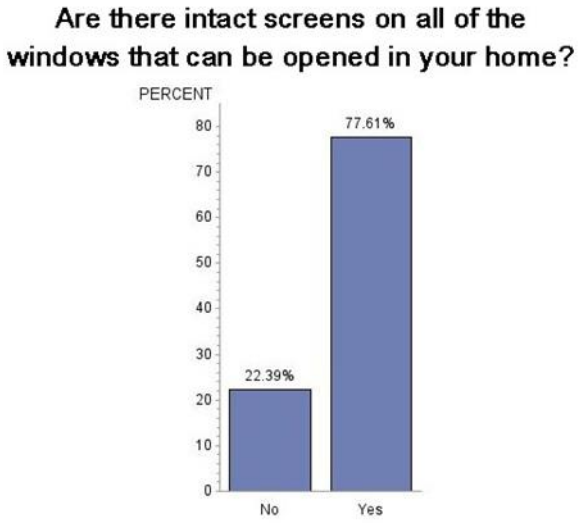
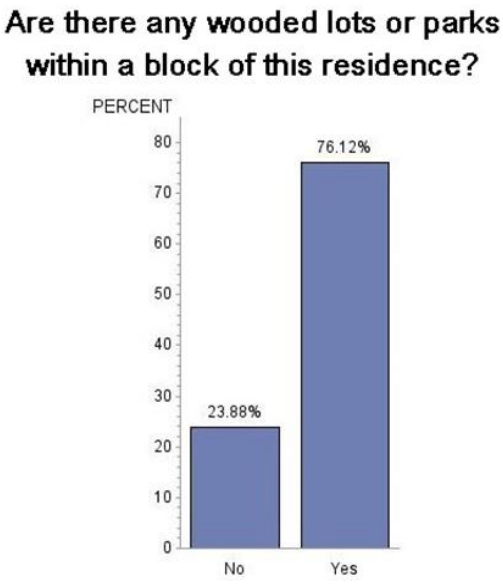


Figure C.3 Histograms of responses to survey questions.







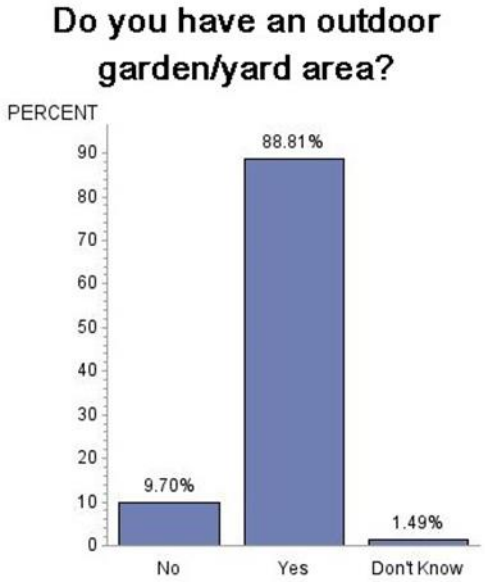
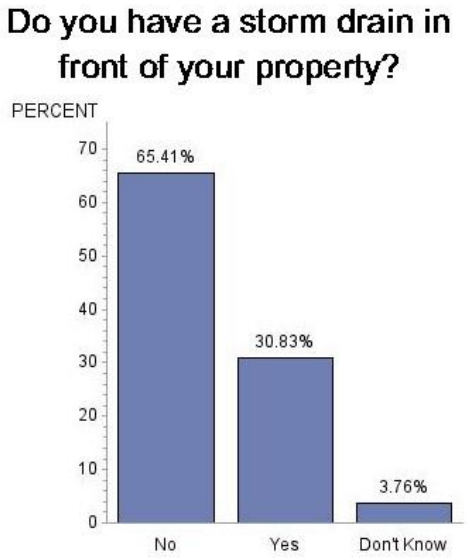
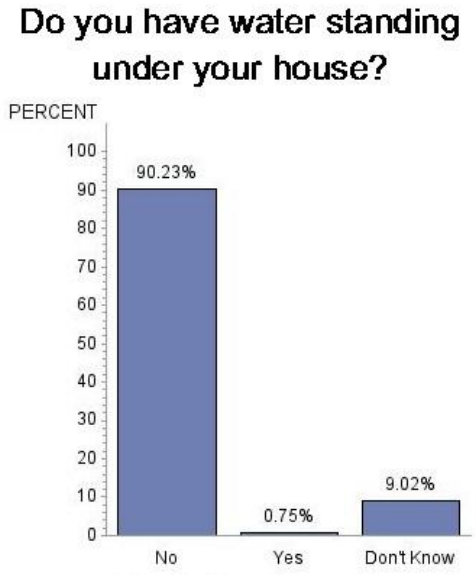
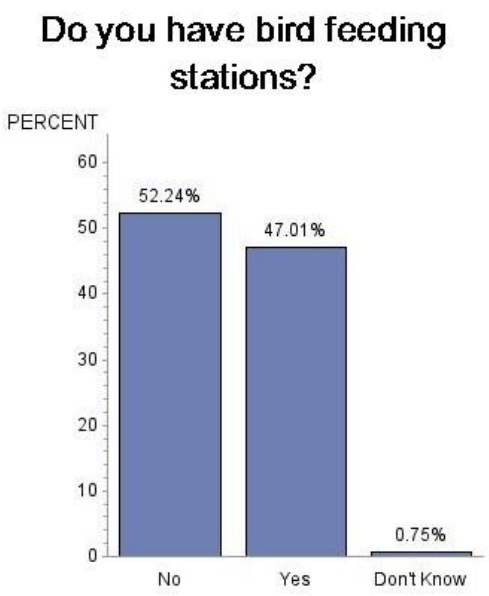


Figure C.3 (continued)







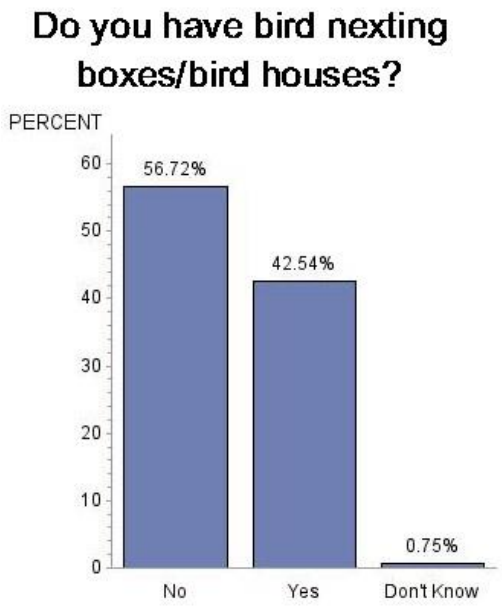


Figure C.3 (continued)

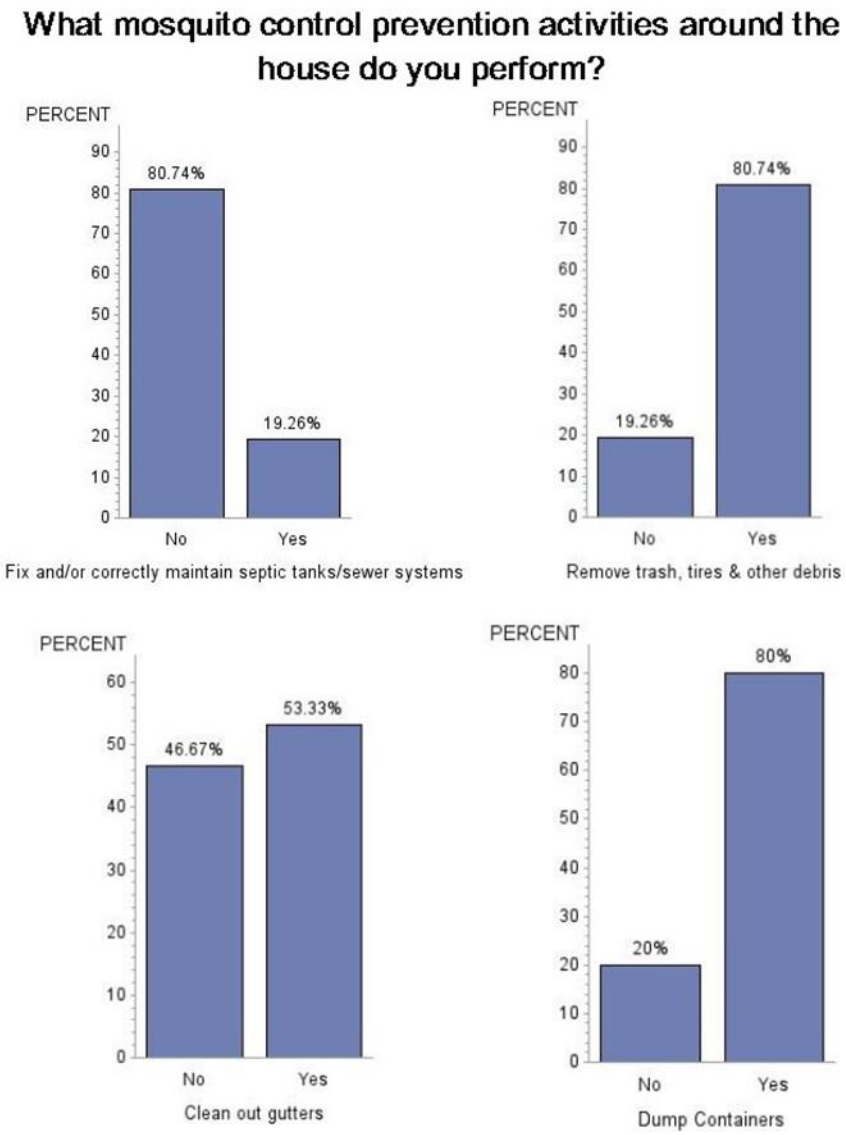
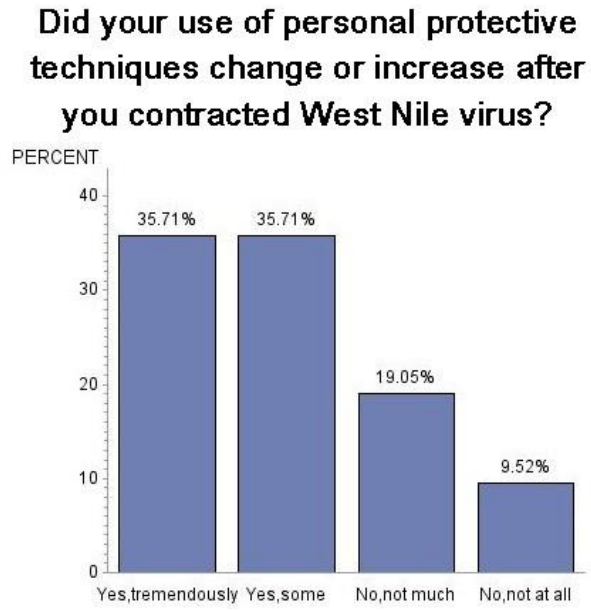


Figure C.3 (continued)



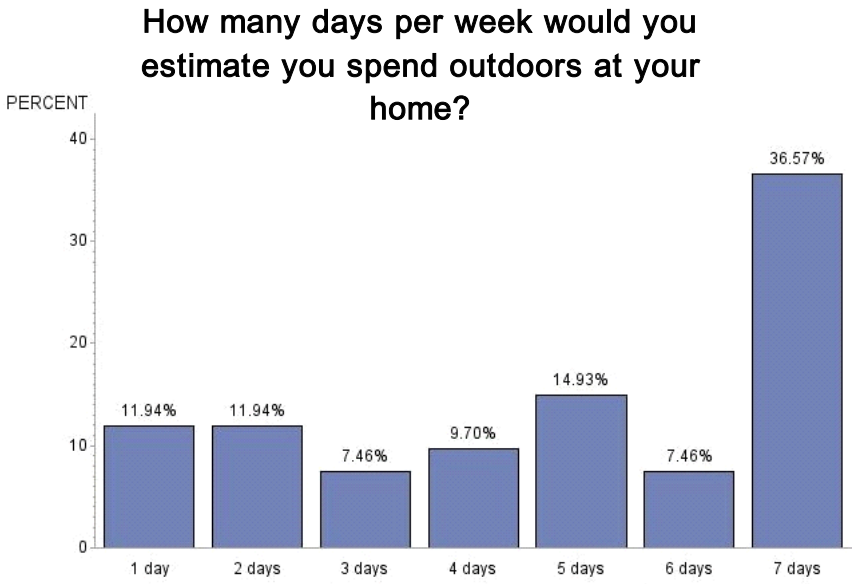
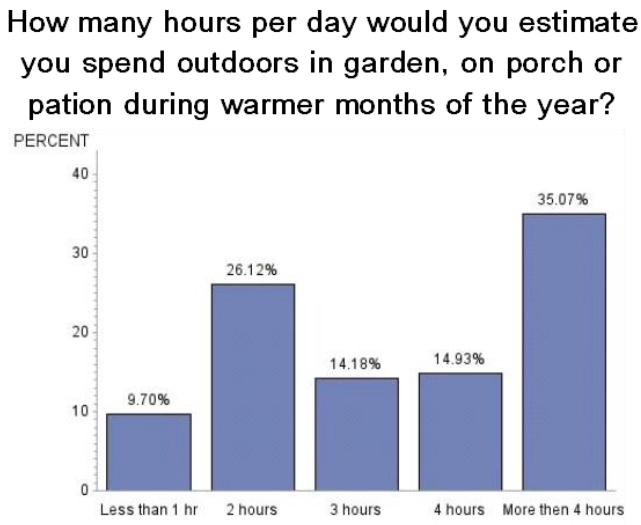


Figure C.3 (continued)



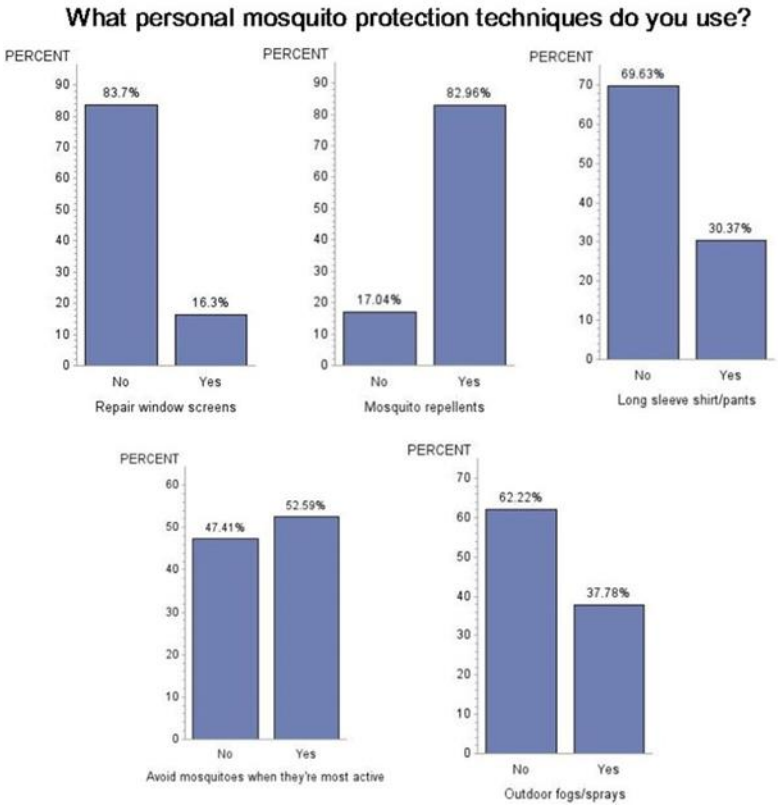
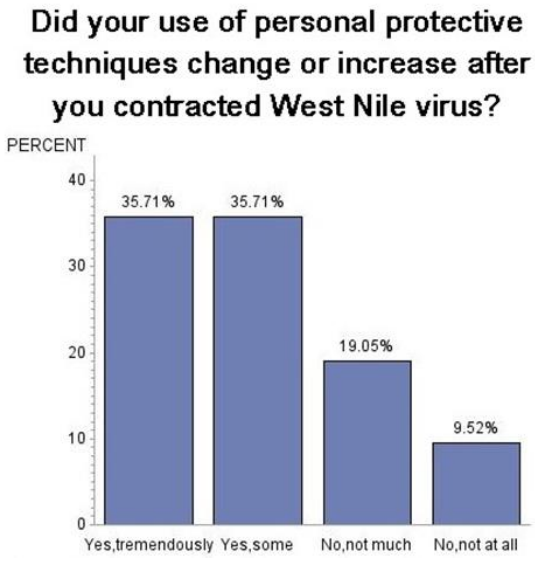


Figure C.3 (continued)



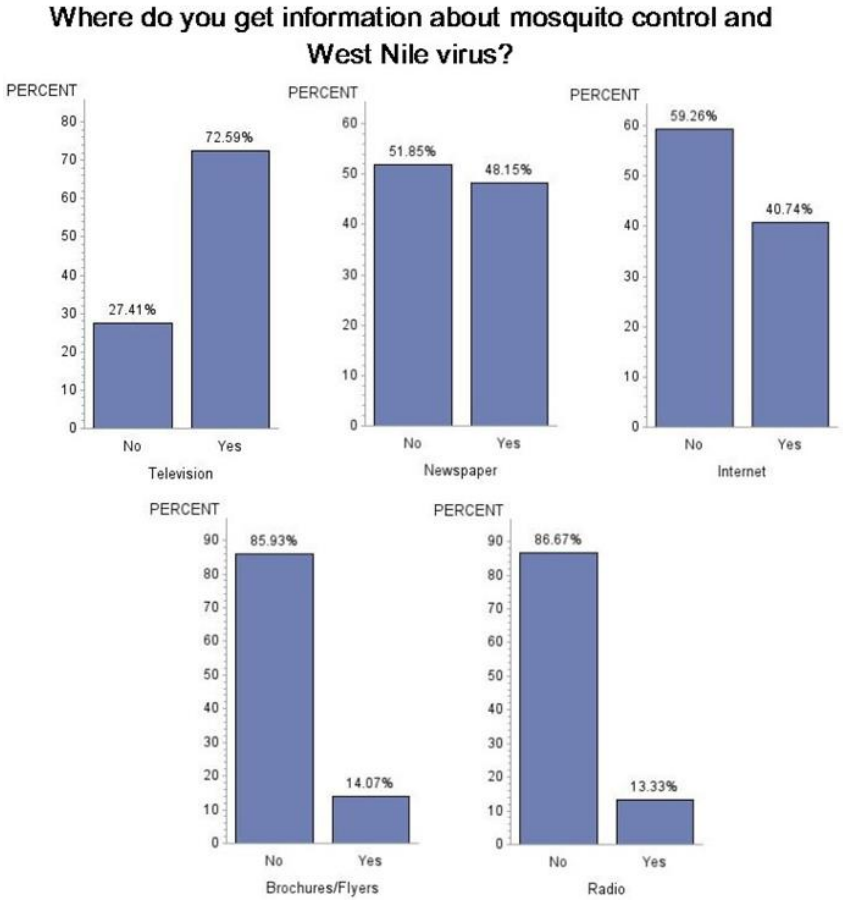


Figure C.3 (continued)

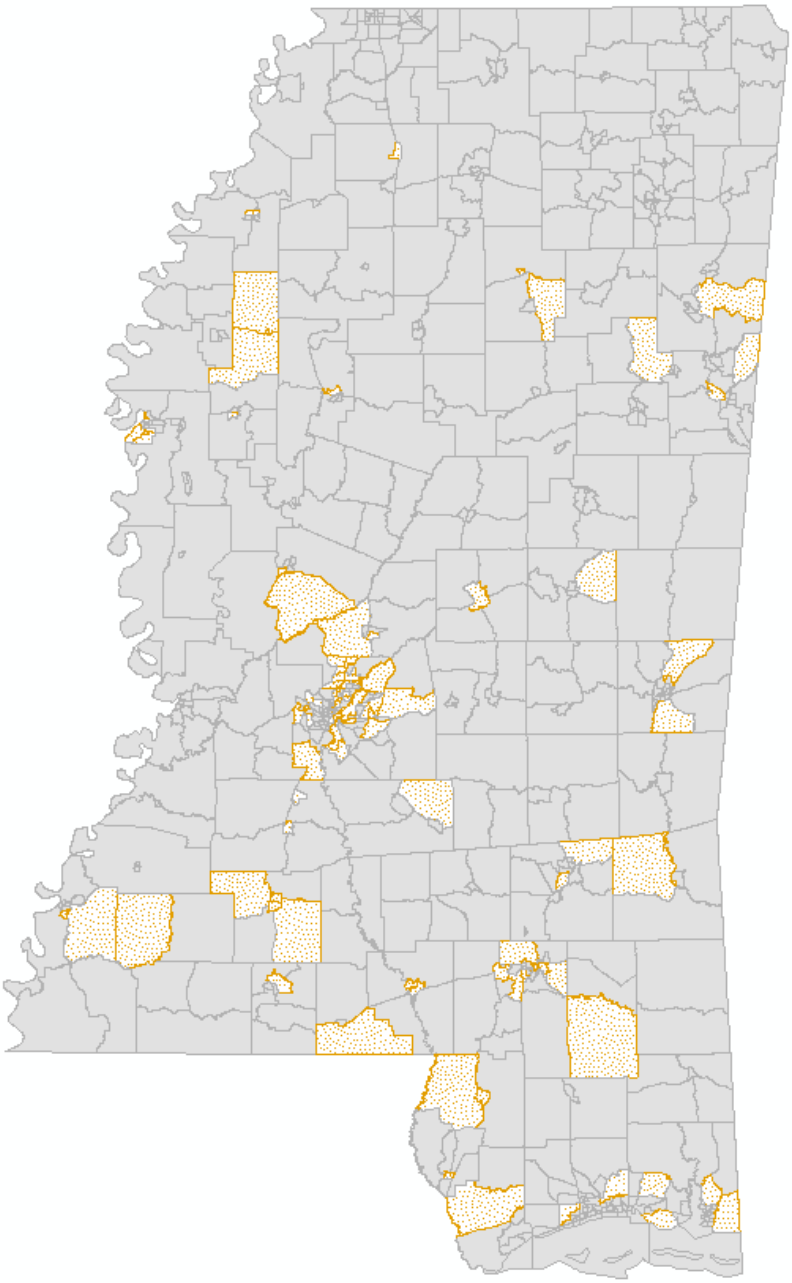


Figure C.4 Mississippi census tracts with human WNV cases 2008-2013. Dotted areas represent census tracts where one or more human WNV cases occurred.

Do you live within the town/city limits?				
q1	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	50	37.31	50	37.31
Yes	84	62.69	134	100.00

Type of housing structure				
q2	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
Single family	124	92.54	124	92.54
Duplex/triplex	3	2.24	127	94.78
Apt. complex	2	1.49	129	96.27
Other	5	3.73	134	100.00

Is air-conditioning used in your house?				
q3	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	1	0.75	1	0.75
Yes	132	98.51	133	99.25
2	1	0.75	134	100.00

If yes, what type of air-conditioning is it?				
q4	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
Central	120	89.55	120	89.55
Window/Wall	14	10.45	134	100.00

Is there an open deck or unscreened porch for your home?				
q5	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	35	26.12	35	26.12
Yes	99	73.88	134	100.00

Are there intact screens on all of the windows that can be opened in your home?				
q6	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	30	22.39	30	22.39
Yes	104	77.61	134	100.00

Figure C.5 WNV patient responses to survey questions related to property description, environmental conditions, personal protection behaviors and homeowner mosquito control activities.

To get rid of wastewater or sewage from your household, is your home connected to:				
q7	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
Municipal sewage system	85	63.43	85	63.43
Individual on-site treatment plant	7	5.22	92	68.66
Septic tank	42	31.34	134	100.00

Home connected to: Other (specify)				
q7_other	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**

Frequency Missing = 134

Are there any wooded lots or parks within a block of this residence?				
q8	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	32	23.88	32	23.88
Yes	102	76.12	134	100.00

During the summer months, do you frequently spend time outdoors at your home, such as in the garden, on a porch or patio?				
q10	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	14	10.45	14	10.45
Yes	119	88.81	133	99.25
2	1	0.75	134	100.00

How many days per week would you estimate that you spend outdoors at your home?				
q9	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
None	2	1.49	2	1.49
one day/week	14	10.45	16	11.94
two days/week	16	11.94	32	23.88
three days/week	10	7.46	42	31.34
four days/week	13	9.70	55	41.04
five days/week	20	14.93	75	55.97
six days/week	10	7.46	85	63.43
seven days/week	49	36.57	134	100.00

Figure C.5 (continued)

How many hours per day would you estimate that you spend outdoors in a garden, on a porch or patio during the summer months of the year?				
q11	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
0	1	0.75	1	0.75
1	12	8.96	13	9.70
2	35	26.12	48	35.82
3	19	14.18	67	50.00
4	20	14.93	87	64.93
5	47	35.07	134	100.00

Do you know if your city or town has a mosquito spray truck?				
q12	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	16	11.94	16	11.94
Yes	103	76.87	119	88.81
3	1	0.75	120	89.55
Don't Know	14	10.45	134	100.00

Television				
q13_1	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	36	26.87	36	26.87
Yes	98	73.13	134	100.00

Newspaper				
q13_2	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	69	51.49	69	51.49
Yes	65	48.51	134	100.00

Internet				
q13_3	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	79	58.96	79	58.96
Yes	55	41.04	134	100.00

Radio				
q13_4	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
No	116	86.57	116	86.57
Yes	18	13.43	134	100.00

Figure C.5 (continued)

Brochures/flyers				
q13_5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	115	85.82	115	85.82
Yes	19	14.18	134	100.00

Dump Containers				
q14_1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	26	19.40	26	19.40
Yes	108	80.60	134	100.00

Clean out gutters				
q14_2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	62	46.27	62	46.27
Yes	72	53.73	134	100.00

Remove trash, garbage, tires & other debris				
q14_3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	25	18.66	25	18.66
Yes	109	81.34	134	100.00

Fix and/or correctly maintain septic tanks/sewer systems				
q14_4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	108	80.60	108	80.60
Yes	26	19.40	134	100.00

Avoid mosquitoes when they're most active				
q15_1	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	63	47.01	63	47.01
Yes	71	52.99	134	100.00

Long sleeve shirt/pants				
q15_2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	93	69.40	93	69.40
Yes	41	30.60	134	100.00

Figure C.5 (continued)

Mosquito repellents				
q15_3	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	22	16.42	22	16.42
Yes	112	83.58	134	100.00

Repair window screens				
q15_4	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	112	83.58	112	83.58
Yes	22	16.42	134	100.00

Outdoor fogs/sprays				
q15_5	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	83	61.94	83	61.94
Yes	51	38.06	134	100.00

Did your use a personal protective techniques change or increase after you contacted West Nile Virus?				
q16	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Yes, tremendously	45	35.71	45	35.71
Yes, some	45	35.71	90	71.43
No, not much	24	19.05	114	90.48
No, not at all	12	9.52	126	100.00

Frequency Missing = 8

Your Property House on pillars (not on concrete slab				
es1_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	87	64.93	87	64.93
Yes	45	33.58	132	98.51
Don't Know	2	1.49	134	100.00

Your Property Water standing under house				
es2_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	120	90.23	120	90.23
Yes	1	0.75	121	90.98
Don't Know	12	9.02	133	100.00

Frequency Missing = 1

Figure C.5 (continued)

Your Property Low lying areas around home/puddles				
es3_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	73	54.89	73	54.89
Yes	53	39.85	126	94.74
Don't Know	7	5.26	133	100.00

Frequency Missing = 1

Your Property Outdoor yard/garden area				
es4_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	13	9.70	13	9.70
Yes	119	88.81	132	98.51
Don't Know	2	1.49	134	100.00

Your Property Abandoned lot				
es5_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	109	81.34	109	81.34
Yes	23	17.16	132	98.51
Don't Know	2	1.49	134	100.00

Your Property Wooded lot/field				
es6_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	51	38.35	51	38.35
Yes	81	60.90	132	99.25
Don't Know	1	0.75	133	100.00

Frequency Missing = 1

Your Property Gutters on home				
es7_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	47	35.34	47	35.34
Yes	84	63.16	131	98.50
Don't Know	2	1.50	133	100.00

Frequency Missing = 1

Your Property Corrugated pipe at base of gutter downspout				
es8_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	75	56.82	75	56.82
Yes	40	30.30	115	87.12
Don't Know	17	12.88	132	100.00

Frequency Missing = 2

Figure C.5 (continued)

Your Property French drain				
es9_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	90	67.16	90	67.16
Yes	19	14.18	109	81.34
Don't Know	25	18.66	134	100.00

Your Property Bird feeding stations				
es10_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	70	52.24	70	52.24
Yes	63	47.01	133	99.25
Don't Know	1	0.75	134	100.00

Your Property Bird nesting boxes/bird houses				
es11_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	76	56.72	76	56.72
Yes	57	42.54	133	99.25
Don't Know	1	0.75	134	100.00

Your Property Birdbath				
es12_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	79	58.96	79	58.96
Yes	53	39.55	132	98.51
Don't Know	2	1.49	134	100.00

Your Property Bushes shrubs				
es13_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	11	8.21	11	8.21
Yes	123	91.79	134	100.00

Your Property Trees				
es14_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	5	3.73	5	3.73
Yes	127	94.78	132	98.51
2	1	0.75	133	99.25
Don't Know	1	0.75	134	100.00

Figure C.5 (continued)

Your Property Overgrown lawn				
es15_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	122	91.73	122	91.73
Yes	11	8.27	133	100.00

Frequency Missing = 1

Your Property Storm drain/storm sewer				
es16_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	87	65.41	87	65.41
Yes	41	30.83	128	96.24
Don't Know	5	3.76	133	100.00

Frequency Missing = 1

Your Property Lake or large pond				
es17_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	99	73.88	99	73.88
Yes	34	25.37	133	99.25
Don't Know	1	0.75	134	100.00

Your Property Stream or open ditch				
es18_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	61	45.52	61	45.52
Yes	70	52.24	131	97.76
Don't Know	3	2.24	134	100.00

Your Property Unmaintained swimming pool				
es19_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	131	97.76	131	97.76
Yes	2	1.49	133	99.25
Don't Know	1	0.75	134	100.00

Your Property Uncovered boat, canoe, kayak				
es20_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	124	92.54	124	92.54
Yes	10	7.46	134	100.00

Figure C.5 (continued)

Your Property Standing water under home from rain/runoff				
es21_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	116	86.57	116	86.57
Yes	8	5.97	124	92.54
Don't Know	10	7.46	134	100.00

Your Property Leaky plumbing under home				
es22_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	122	91.04	122	91.04
Yes	3	2.24	125	93.28
Don't Know	9	6.72	134	100.00

Your Property Rain barrels				
es23_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	124	92.54	124	92.54
Yes	7	5.22	131	97.76
Don't Know	3	2.24	134	100.00

Your Property Potted plants				
es24_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	29	21.64	29	21.64
Yes	105	78.36	134	100.00

Your Property Kiddy pool				
es25_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	126	94.03	126	94.03
Yes	6	4.48	132	98.51
Don't Know	2	1.49	134	100.00

Your Property Kids toys or lawn ornaments				
es26_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	98	73.13	98	73.13
Yes	35	26.12	133	99.25
Don't Know	1	0.75	134	100.00

Figure C.5 (continued)

Your Property Pet dishes				
es27_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	78	58.21	78	58.21
Yes	56	41.79	134	100.00

Your Property Ornamental pond without fish				
es28_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	127	94.78	127	94.78
Yes	7	5.22	134	100.00

Your Property Watering cans/buckets				
es29_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	89	66.42	89	66.42
Yes	44	32.84	133	99.25
Don't Know	1	0.75	134	100.00

Your Property Old tires				
es30_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	119	88.81	119	88.81
Yes	13	9.70	132	98.51
Don't Know	2	1.49	134	100.00

Your Property Any item covered with a tarp				
es31_YP	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	100	74.63	100	74.63
Yes	33	24.63	133	99.25
Don't Know	1	0.75	134	100.00

Figure C.5 (continued)