Use of the VectorTest for Advanced Warning of Human West Nile Virus Cases in Mississippi

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Abstract West Nile virus (WNV) continues to persist in Mississippi; 2012 was the worse year for human infections, with a total of 247 reported human cases and five deaths. Public health officials are keenly interested in ways to detect WNV in advance in their jurisdictions, so they can implement appropriate and timely mosquito control in affected areas. A total of 40,312 female *Culex quinquefasciatus* mosquitoes were collected by gravid traps in Mississippi in 2013 and 2014 and tested by VectorTest, a rapid immunochromatographic assay ("dip-stick" test) that is a highly specific and effective rapid threat assessment tool. This study evaluated if and to what extent VectorTest could provide advanced warning of impending human WNV cases in a specific area. These data were examined with regard to date of onset of human WNV cases to determine the predictive value of VectorTest for WNV activity. Both years, positive mosquito pools appeared before the vast majority (87.2%) of reported human cases. Overall, in 27 out of 37 human WNV cases (73.0%) occurring in our study sites, there was an average advanced warning of 26 days (range 11–53 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 can inform public health and mosquito control personnel of WNV activity in an area and of impending human cases.

Introduction

West Nile virus (WNV) is a mosquito-borne, enveloped single-stranded, positive-sense RNA virus belonging to the *Flaviviridae* family of viruses (Tesh & Solomon, 2011). WNV was first discovered in the Western Hemisphere in 1999 in New York City, New York, where there were a total of 59 cases and seven deaths (Asnis, Conetta, Teixeira, Waldman, & Sampson, 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.

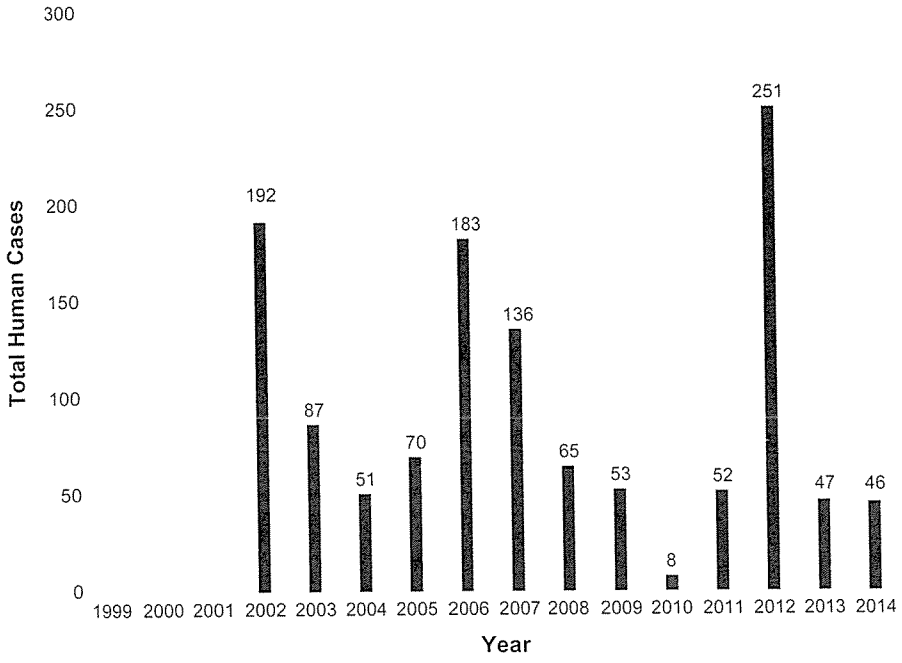
There are no known specific treatments for WNV and the patient is generally treated with only supportive care. WNV was first documented in Mississippi in humans in July 2002 (Centers for Disease Control and Prevention [CDC], 2002) and by the end of 2002, Mississippi had a total of 192 WNV cases with 162 of those resulting in serious encephalitis; there were 12 deaths (CDC, 2002). In the decade since its introduction into Mississippi, WNV has continued to persist statewide. The year 2012 was the 10th anniversary of the introduction of WNV in Mississippi and proved to be the worse year yet for human infections, with a total of 247 human cases and five deaths (Mississippi State Department of Health, 2014) (Figure 1).

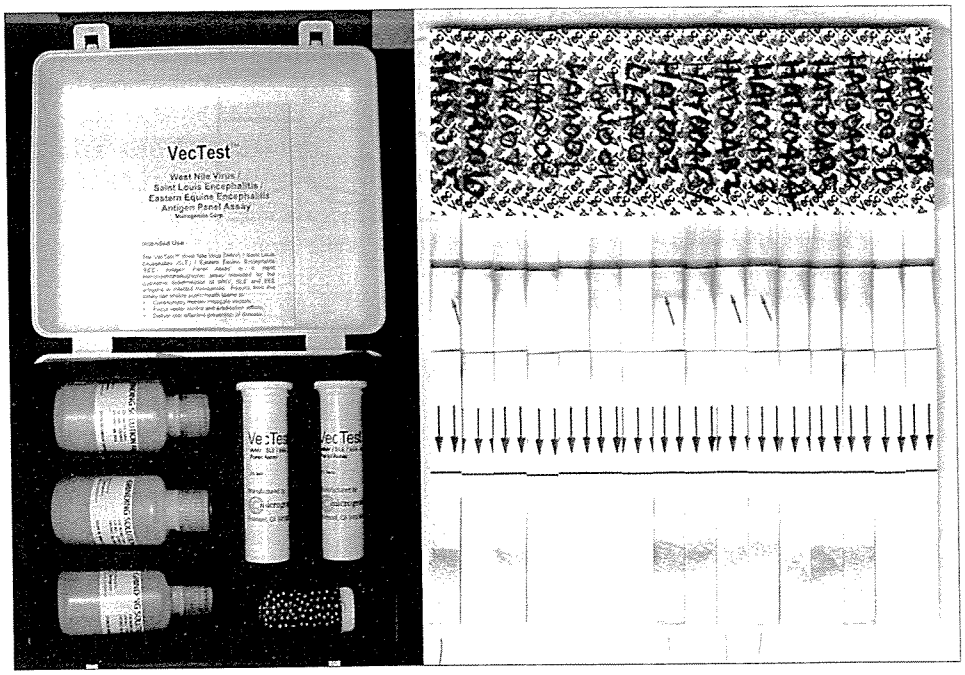
Public health officials are keenly interested in ways to detect WNV in mosquitoes and sentinel animals, with the ultimate goal of implementing appropriate and timely mosquito control in affected areas (Goddard, 2013; Gu & Novak, 2004). Some studies have utilized landscape ecology and/or weather and demographic data to try to predict WNV activity (Gu, Unnasch, Katholi Lampman, & Novak, 2008; Manore et al. 2014; Young, Tullis, & Cothren, 2013).

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), Vero cell plaque assays, and viral antigen assays. The VectorTest is a rapid immunochromatographic assay ("dip-stick" test) intended for the qualitative determination of WNV antigens in infected mosquitoes (see photo at right). 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

FIGURE 1

Historical Human West Nile Virus Cases by Year, Mississippi





VectorTest (previously known as VecTest) kit (left) and test strips (right).

more cost-effective to state and local surveillance programs. Although the VectorTest might miss some positives as compared with PCR assays, VectorTest 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).

Several studies have attempted to link mosquito surveillance data with human WNV infections (Ginsberg, Rochlin, & Campbell, 2010; Kilpatrick & Pape, 2013; Liu et al., 2009), and 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 in July (Ginsberg et al., 2010). 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 (Kilpatrick & Pape, 2013; Kulasekera et al., 2001). In addition, minimum infection rates (MIR) and vector mosquito abundance can be combined into a "vector index" that is a good indicator of human WNV risk, a method advocated by CDC (Chung et al., 2013; Jones et al., 2011; Kwan et al., 2012).

The purpose of this study was to determine if, and to what extent, environmental health personnel can use mosquito testing to acquire advanced warning of impending human WNV cases in a specific area.

Methods

Collection Sites

Nine areas throughout Mississippi were selected for mosquito sampling. Three of the areas consisted of more than one town/city in one geographic location; all other areas were cities by themselves (Figure 2). The Golden Triangle collection area included the towns of West Point, Starkville, Columbus, and Louisville. The Jackson Metro area included the 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 hot spots 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 *Culex quinquefasciatus* mosquitoes, and thus were considered favorable for WNV activity.

Mosquito Collections

Local county mosquito control staff, health department personnel, and health department interns were tasked with operating CDC Gravid Traps (see photo at bottom right) at the selected sites from approximately June 1–September 15 each year. These dates varied somewhat due to health department administrative and budget issues.

All personnel involved in trapping received appropriate training prior to project start. 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).

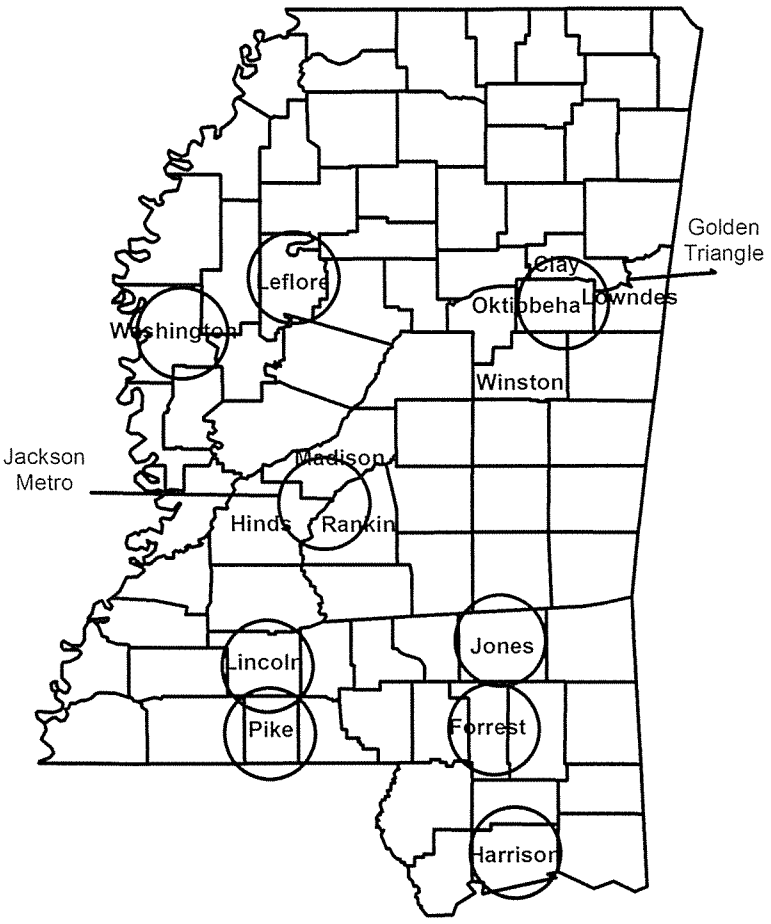
Traps were set weekly at each site in late afternoon and retrieved the following morning, unless inclement weather prevented retrieval. Each trap was powered by one 6 V, 10 amp rechargeable gelled-electrolyte battery. Traps were baited with a fish-oil emulsion mixture containing approximately three ounces 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 mosquitoes collected in one trap night, which can be subdivided into smaller groups called "pools" for testing. In this study, due to financial constraints, no fewer 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.

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. Test strips were read within 30 minutes of the assay. Any strips with indistinct bands were classified as "maybe positive" samples (see photo on page 21). For outside quality assurance, all mosquito pools that tested positive and the "maybe" samples were sent to CDC, Division of Vector-Borne Diseases, Arboviral Diseases Branch in Fort Collins, Colorado, for follow-up testing and confirmation with RT-PCR using previously described methods (Burkhalter et al., 2006; Ryan et al., 2003).

FIGURE **2**

Nine Mosquito Collection Sites Throughout the State of Mississippi

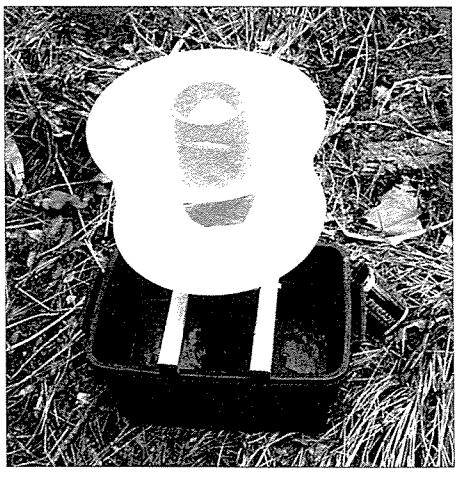


Calculation of MIR

We calculated MIR by dividing the total number of WNV-positive mosquito pools in each site by the total number of mosquitoes tested. MIR is expressed as the number of infected mosquitoes divided by total number tested multiplied by 1,000. The MIR is based on the assumption that infection rates are low and that only one mosquito is positive in a pool (CDC, 2013).

WNV Human Case Data

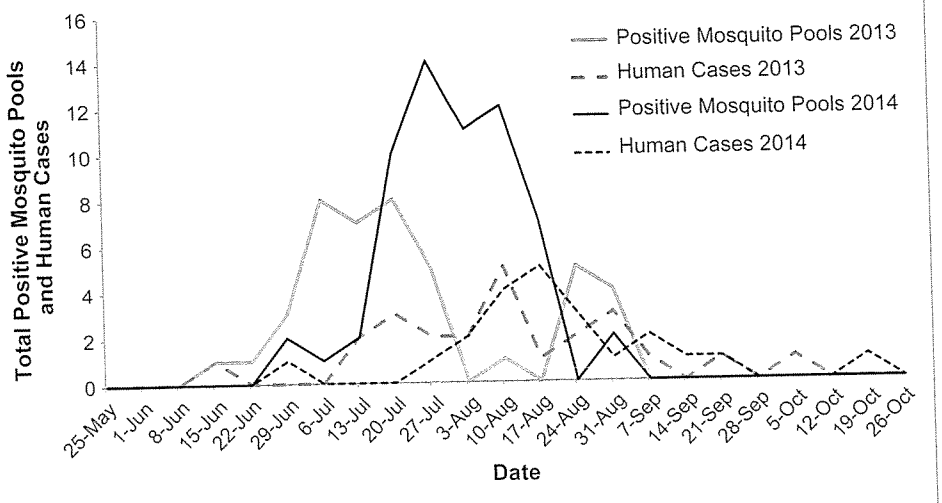
Human WNV cases were determined using the Mississippi State Department of Health (MSDH) EpiTracks system. These human WNV cases included clinical cases confirmed



A typical gravid trap set up.

FIGURE 3

Overall Pattern of Dates of Mosquito Infection and Human West Nile Virus Symptom Onset for All Sites Combined, 2013–2014



by the MSDH Public Health Laboratory and/or CDC, 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).

Results and Discussion

A total of 40,312 (16,259 in 2013 and 24,053 in 2014) *Cx. quinquefasciatus* mosquitoes were collected in the nine sites over the 2-year period with an average of 72.6 per trap (77.3 in 2013 and 66.91 in 2014) ranging from 5–900 in 2013 and 10–900 in 2014. During 2014, no collections were made from Greenwood due to contracting issues; therefore there were only eight sites that year. The overall MIR over the 2-year period ranged from 0–9.9 out of 1,000 with Hattiesburg having the highest MIR and Biloxi/Gulfport, Brookhaven, and the Golden Triangle areas having the lowest MIR. 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 are likely due to the presence of a well-run, publicly-funded, integrated mosquito control program in that area.

There were 18 confirmed human WNV cases in the nine collection areas during 2013 and 21 cases in eight 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). Thirty-seven of these 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. Overall, in 27 out of 37 human WNV cases (73.0%) occurring in our study site, there was an average advanced warning of 26 days (range 11–53 days) as indicated by positive mosquito collections near the patient's home, assuming that the patient contracted WNV at or near home.

As for quality assurance and outside confirmation of our results by CDC, 34 out of 36 of VectorTest positive samples (94.4%) were also WNV positive when retested using RT-PCR. This means two samples were false positives using VectorTest. The cause of this discrepancy is unknown. Of the seven "maybe" samples submitted from 2013, all but one was positive by RT-PCR (there appeared to be a faintly visible positive line with the VectorTest). As for 2014, a total of 63 out of 67 of VectorTest positive samples (94.0%) were WNV positive when retested using 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 titer to accurately confirm positive for WNV. Of the five "maybe" samples submitted from 2014, four were positive by RT-PCR and one fell into the questionable group after RT-PCR testing.

This operational health department study, although somewhat limited, reveals that mosquito sampling and testing can inform public health and mosquito control personnel of WNV activity in an area and therefore, of impending human cases.

In our study, 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 a relatively inexpensive and less labor-intensive product, in this case VectorTest, is more than adequate for health departments or mosquito control agencies that might not have sophisticated and expensive molecular analysis capability.

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