Centers for Disease Control and Prevention
Division of Vector-Borne Diseases
Promote health and quality of life by preventing and controlling vector-borne diseases

West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control

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- National Association of City and County Health Officials (NACCHO)
- Council of State and Territorial Epidemiologists (CSTE)
- Association of State and Territorial Health Officials (ASTHO)
- Association of Public Health Laboratories (APHL)
- American Mosquito Control Association (AMCA)

Foreword

As West Nile virus (WNV) spread and became established across the United States following its first identification in New York City in 1999, the responses of all levels of the public health system have resulted in a detailed understanding of WNV transmission ecology and epidemiology as well as development of systems and procedures to reduce human risk. This includes an expanded capacity to diagnose and monitor WNV infections in humans, measure WNV transmission activity in vector mosquitoes, and implement effective WNV control programs. These guidelines, which update the third revision released in 2003, incorporate this new knowledge with the goal of providing guidance to health departments and other public health entities in monitoring and mitigating WNV risk to humans.

Human disease surveillance provides an ongoing nationwide assessment of the human impact of WNV, and over the past decade has demonstrated where WNV incidence and total disease burden are greatest. However, human disease surveillance, by itself, is limited in its ability to predict the large focal outbreaks that have come to characterize this disease. These outbreaks typically intensify over as little as a couple of weeks; however, human case reports are lagging indicators of risk since case reports occur weeks after the time of infection. Thus, environmental surveillance – monitoring enzootic and epizootic WNV transmission in mosquitoes and birds – forms a timelier index of risk, and is an important cornerstone for implementing effective WNV risk reduction efforts. Research and operational experience shows that increases in WNV infection rates in mosquito populations can provide an indicator of developing outbreak conditions several weeks in advance of increases in human infections.

Communities that have a history of WNV, particularly metropolitan areas with large human populations at risk, should implement comprehensive, integrated vector management (IVM) programs that incorporate monitoring mosquito abundance and infection rates. Mosquito-based WNV surveillance programs should use strategies that assure data are comparable over time and space, and are designed to detect trends in WNV transmission levels. Programs should enlist quantitative indicators such as the WNV infection rate or vector index to represent WNV transmission activity in mosquito populations. Programs must be sustainable over the long term in order to provide sufficient information to link surveillance indicators with the degree of human risk. Consistency also requires that mosquito collections be repeated at regular (weekly) intervals over the course of the transmission season, and that collections are made at fixed collecting sites. Only through maintaining consistency can monitoring programs provide information useful in crafting thresholds to support decisions about vector control efforts and other interventions. Other surveillance modalities, such as sentinel chickens and dead bird surveillance, may be a valuable adjunct to mosquito-based surveillance in quantifying epizootic activity in some settings.

IVM programs must be proactive and make plans in advance for addressing increasing levels of WNV risk. The objective of IVM is to implement control measures sufficient to maintain mosquito abundance below levels that result in high risk of WNV transmission to humans. By establishing action thresholds based on the abundance of WNV-infected vector mosquitoes, IVM programs can monitor risk and the effectiveness of their programs, and implement more directed vector control efforts as needed. IVM implies that all of the tools available for managing mosquito populations should be considered for use as needed to maintain vector populations at low levels. Source reduction and larval control activities can be effective in maintaining low vector abundance, but adult mosquito control efforts through ground or aerially applied pesticides complement proactive vector management programs and should not be relegated to the status of a “last resort” measure to be used only during outbreaks. Aggressive and

timely efforts to reduce the number of infected adult mosquitoes will optimally impact human WNV case incidence when environmental surveillance indicates substantial WNV epizootic activity or when many human cases occur early in the season (e.g., June or July.)

This document provides guidance for communities and public health agencies developing new programs or enhancing existing WNV management programs. The CDC Division of Vector-Borne Diseases is available to provide additional consultation and technical assistance (by phone: 970-261-6400 or email: dvbid2@cdc.gov).

Introduction

Ten years have passed since the 2003 publication of the 3rd edition of "West Nile virus in the United States: Guidelines for surveillance prevention and control". At that time, only 4 years since West Nile virus (WNV) was first detected in New York City, the virus had already established itself across approximately the eastern half of the country and produced the largest epidemic of arboviral encephalitis ever experienced in the United States. Knowledge about WNV epidemiology and transmission ecology was expanding rapidly, but numerous gaps remained in our understanding of how this relatively new exotic disease would affect public health, what monitoring practices would provide the best indicators of human risk, and what interventions would be most effective in reducing human infections. Thus, large portions of the WNV Guidelines 3rd edition were predicated on relatively limited research and operational experience, and were dedicated to identifying and prioritizing specific basic and operational research directions.

Since that time, WNV has expanded to the point that it can now be found in all 48 contiguous states and has produced two additional, large nationwide epidemics in 2003 and 2012. Also, considerable new information about WNV epidemiology, ecology and control has been generated since 2003. The objective of this 4th edition of the WNV guidelines is to consolidate and describe this information and describe how these new findings can be used to better monitor WNV and mitigate its public health impact.

This document was produced through a comprehensive review of the published literature related to WNV epidemiology, diagnostics, transmission ecology, environmental surveillance, and vector control. The publications were reviewed for relevance to developing operational surveillance and control programs, and selected for inclusion in a draft document by a technical development group of CDC subject matter experts. Numerous stakeholder groups were requested to review the document. Comments and additional material provided by National Association of Vector-Borne Disease Control Officials (NAVCO), National Association of City and County Health Officials (NACCHO), Council of State and Territorial Epidemiologists (CSTE), Association of State and Territorial Health Officials (ASTHO), Association of Public Health Laboratories (APHL), and American Mosquito Control Association (AMCA) were incorporated to produce this document.

We view the recommendations contained in these guidelines as the best that can be derived from the currently available information, and will provide updates as new information about WNV epidemiology, ecology, or intervention becomes available.

West Nile Virus Epidemiology and Ecology

WNV, a mosquito-transmitted member of the genus Flavivirus in the family *Flaviviridae*, was discovered in northwest Uganda in 1937 (Smithburn et al. 1940) but was not viewed as a potentially important public health threat until it was associated with epidemics of fever and encephalitis in the Middle East in the 1950's (Taylor et al. 1956, Paz 2006). In the following years, WNV was associated with sporadic outbreaks of human disease across portions of Africa, the Middle East, India, Europe and Asia (Hubalek and Halouzka 1999). In the mid to late 1990's outbreaks occurred more frequently in the Mediterranean Basin and large outbreaks occurred in Romania and the Volga delta in southern Russia (Hayes et al. 2005).

The first domestically-acquired human cases of WNV disease in the Western Hemisphere were detected in New York City in 1999 (Nash et al. 2001). WNV rapidly spread during the following years, and by 2005 had established sustained transmission foci in much of the hemisphere with an overall distribution that extended from central Canada to southern Argentina (Gubler 2007). WNV transmission persists across this large, ecologically-diverse expanse, and as a result this virus is recognized as the most widely distributed arbovirus in the world (Kramer et al. 2008).

WNV has become enzootic in all 48 contiguous United States and evidence of transmission in the form of infected humans, mosquitoes, birds, horses, or other mammals has been reported from 96% of U.S. counties. This extensive distribution is due to the ability of WNV to establish and persist in the wide variety of ecosystems present across the country. WNV has been detected in 65 different mosquito species in the U.S. (CDC 2012), though it appears that only a few *Culex* species drive epizootic and epidemic transmission. The most important vectors are *Cx. pipiens* in the northern half of the country, *Cx. quinquefasciatus* in the southern states, and *Cx. tarsalis* in the western states where it overlaps with the *Cx. pipiens and quinquefasciatus* (Fig 1.) (Andreadis et al. 2004, Kilpatrick et al. 2006a, Godsey et al. 2010). However, the population structure of *Culex pipiens* and *Cx. quinquefasciatus* is more complex than indicated in Fig. 1 as these species readily hybridize and produce a stable hybrid zone across the United States. Barr (1957) set the limits of the hybrid zone at 36^{O} N and 39^{O} N based on measurements of the male genitalia. Subsequent work using microsatellites (Huang et al. 2008, Edillo et al. 2009, Kothera et al. 2009, Kothera et al. 2013, Savage and Kothera 2012) and other molecular markers (Huang et al. 2011) indicates that the hybrid zone extends farther north and south than suggested by Barr (1957). In the middle latitudes of the US, both nominal species and hybrids may be present and are commonly reported as *Cx. pipiens* complex mosquitoes (Savage et al. 2007, Savage and Kothera 2012). The implications of *Cx. pipiens – Cx. quinquefasciatus* population genetics any hybridization patterns for WNV transmission are not well understood.

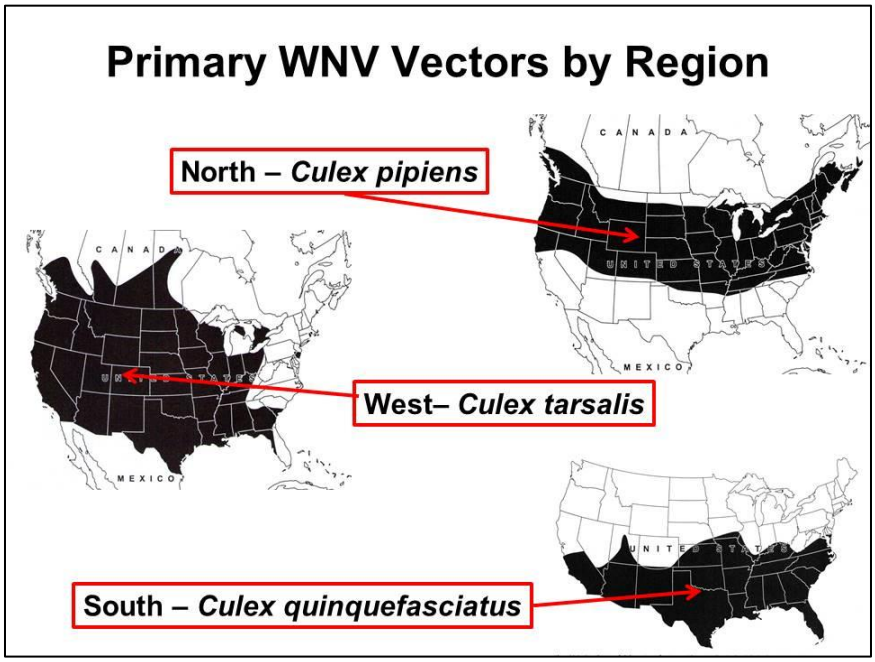

Figure 1. Approximate geographic distribution of the primary WNV vectors, *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. tarsalis* (modified from Darsie and Ward 2005).

Culex salinarius has been identified as an important enzootic and epidemic vector in the northeastern U.S. (Anderson et al. 2004, 2012, Molaei et al. 2006). Other mosquito species including *Cx. restuans, Cx. nigripalpus* and *Cx. stigmatosoma* may contribute to early season amplification or serve as accessory bridge vectors in certain regions, but their role is less well understood (Kilpatrick et al. 2005). WNV has been detected in hundreds of bird species in the United States (CDC 2012). However, relatively few species function as primary amplifiers of the virus, and a small subset of bird species may significantly influence WNV transmission dynamics locally (Hamer et al. 2009). For example, the American robin (*Turdus migratorious*) can play a key role as amplifier host, even in locations where it is present in relatively low abundance (Kilpatrick et al. 2006b).

As a result of this extensive distribution in the U.S., WNV is now the most frequent cause of arboviral disease in the country. Since 1999, WNV disease cases have been reported from all 48 contiguous states and two-thirds of all U.S. counties. Though widely distributed, WNV transmission is temporally and spatially heterogeneous. Human WNV disease cases have been reported during every month of the year in the United States, but as is characteristic of zoonotic arboviruses in temperate climates, intense transmission is limited to the summer and early fall months; 94% of human cases have been reported from July through September (CDC 2010) and approximately two-thirds of reported cases occurred during a 6-week period from mid-July through the end of August. At a national level, the annual incidence and number of cases reported have varied dramatically since 1999 (CDC 2010, CDC 2010a, CDC 2011, CDC 2012). Weather, especially temperature, is an important modifier of WNV transmission, and has been correlated with increased incidence of human disease at regional and national scales (Soverow et al. 2009), and likely drives the annual fluctuations in numbers of cases reported at the national level. However, WNV epidemiology is characterized by focal and sometimes intense outbreaks (CDC 2010). Epidemiological data gathered since 1999 demonstrate regions in the United States with recurring high levels of WNV transmission and risk to humans (Fig. 2). High average annual incidence of WNV disease occurs in the West Central and Mountain regions (CDC 2010), with the highest cumulative incidence of infection occurring in the central plains states (i.e., South Dakota, Wyoming and North Dakota) (Petersen et al. 2012). The greatest disease burden occurs where areas of moderate to high incidence intersect metropolitan counties with correspondingly high human population densities.

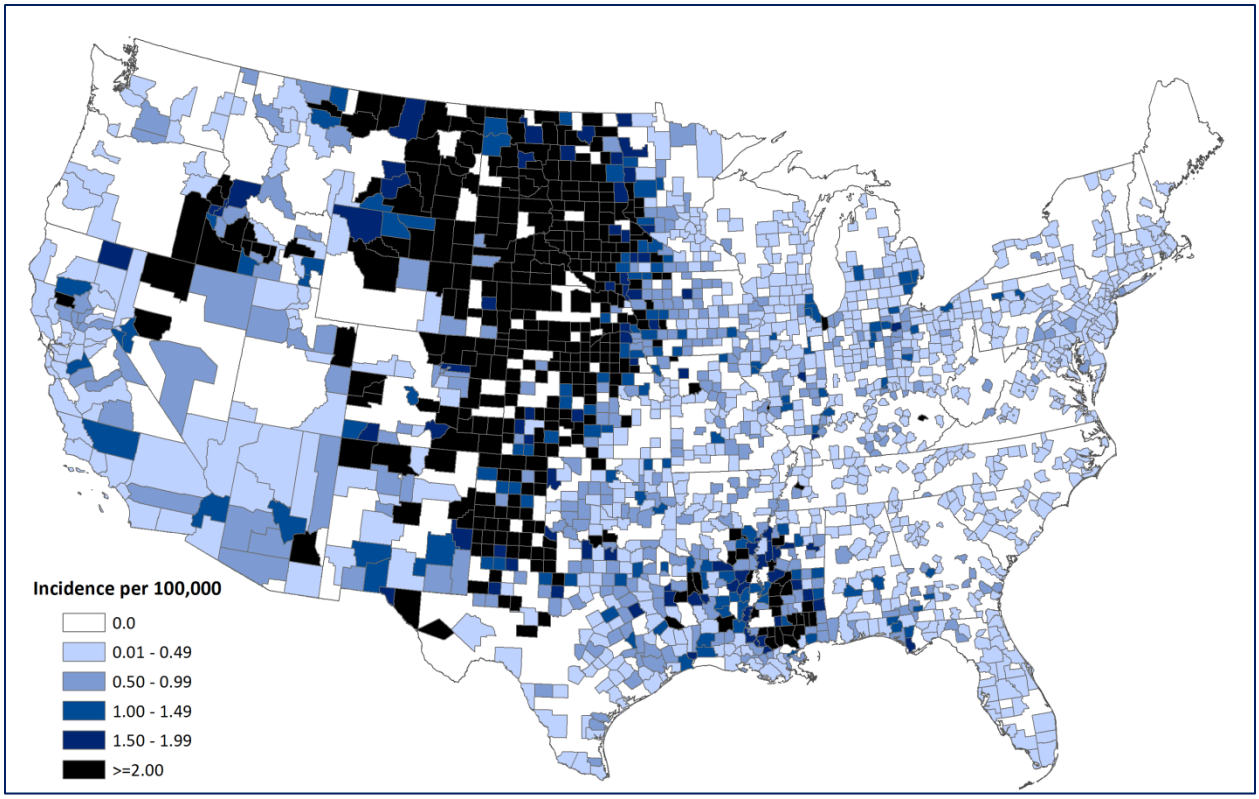

Figure 2. Average annual incidence of West Nile Virus neuroinvasive disease, 1999-2012.

Limits to Prediction – The Need for Surveillance

WNV outbreaks have been associated on a local level with a variety of parameters including urban habitats in the Northeast and agricultural habitats in the western United States (Bowden et al. 2011), rural irrigated landscapes (DeGroote and Sugumaran 2012), increased temperature (Hartley et al. 2012), specific precipitation patterns, several socioeconomic factors such as housing age and community drainage patterns (Ruiz et al. 2007), per capita income (DeGroote and Sugumaran 2012), and neglected swimming pool density (Reisen et al. 2008, Harrigan et al. 2010). Despite these documented associations with a variety of biotic and abiotic factors, and recognition that certain regions experience more frequent outbreaks and higher levels of human disease risk, no models have been developed to provide long-term predictions of how and where these factors will combine to produce outbreaks. The unpredictable nature of WNV outbreaks necessitates the establishment and maintenance of surveillance systems capable of detecting increases in WNV transmission activity and the ability to respond to the surveillance data with effective, disease-reducing interventions. Such surveillance and control programs can be costly to maintain. However it is important that communities with large human populations in areas with documented WNV risk establish and maintain surveillance for human cases and effective integrated vector management programs that incorporate environmental surveillance components capable of providing indicators predictive of human risk.

This document provides guidance for developing systems to 1) Monitor WNV enzootic and epizootic transmission activity as indicators of human risk; 2) Maintain surveillance for human infections and

disease to monitor trends in WNV disease burden and clinical presentation; and 3) Implement prevention and control programs that reduce community level risk by managing vector mosquito populations and that reduce individual risk by promoting effective personal protection measures.

Surveillance

Objectives of WNV Surveillance

WNV surveillance consists of two distinct, but complementary activities. Epidemiological surveillance measures WNV human disease to quantify disease burden and identify seasonal, geographic, and demographic patterns in human morbidity and mortality (Lindsey et al. 2008, CDC 2010). Environmental surveillance monitors local WNV activity in vectors and non-human vertebrate hosts in advance of epidemic activity affecting humans.

In addition to monitoring disease burden and distribution, epidemiological surveillance has been instrumental in characterizing clinical disease presentation and disease outcome, as well as identifying high risk populations and factors associated with serious WNV disease (Lindsey et al. 2012). Epidemiological surveillance has also detected and quantified alternative routes of WNV transmission to humans, such as contaminated blood donations and organ transplantation (Pealer et al. 2003, Nett et al. 2012). Epidemiological and environmental surveillance for WNV was facilitated by development and implementation of ArboNET, the national arbovirus surveillance system (Lindsey et al. 2012). ArboNET was developed in 2000 as a comprehensive surveillance data capture platform to monitor WNV infections in humans, mosquitoes, birds, and other animals as the virus spread and became established across the country. This comprehensive approach was essential to tracking the progression of WNV activity and remains a significant source of data adding to our understanding of the epidemiology and ecology of WNV.

In the absence of effective human WNV vaccines, preventing disease in humans depends on application of measures to keep infected mosquitoes from biting people. A principal objective of WNV environmental surveillance is to quantify the intensity of virus transmission in a region in order to provide a predictive index of human infection risk. This risk prediction, along with information about the local conditions and habitats that produce WNV vector mosquitoes, can be used to inform an integrated vector management program and the associated decisions about implementing prevention and control interventions (Nasci 2013).

Though epidemiological surveillance is essential for understanding WNV disease burden, utilizing human case surveillance by itself is insufficient for predicting outbreaks (Reisen and Brault 2007). WNV outbreaks can develop quickly, with the majority of human cases occurring over a few weeks during the peak of transmission (CDC 2010). The time from human infection to onset of symptoms, to diagnosis and reporting can be several weeks or longer. As a result, human WNV case reports lag well behind the transmission from mosquitoes that initiated the infection. By monitoring WNV infection prevalence in mosquito vectors and incidence in non-human vertebrate hosts, and comparing these indices to historical environmental and epidemiological surveillance data, conditions associated with increasing human risk can be detected 2-4 weeks in advance of human disease onset (Kwan et al. 2012a). This provides additional lead time for critical vector control interventions and public education programs to be put in place. The following sections describe the elements of epidemiological and environmental WNV surveillance and how they may be used to monitor and predict risk and to trigger interventions.

Human Surveillance

Routes of Transmission

WNV is transmitted to humans primarily through the bite of infected mosquitoes (Campbell et al. 2001). However, person-to-person transmission can occur through transfusion of infected blood products or solid organ transplantation (Pealer et al. 2003, Iwamoto et al. 2003). Intrauterine transmission and probable transmission via human milk also have been described but appear to be uncommon (O'Leary et al. 2006, Hinckley et al. 2007). Percutaneous infection and aerosol infection have occurred in laboratory workers, and an outbreak of WNV infection among turkey handlers also raised the possibility of aerosol transmission (CDC 2002, CDC 2003a).

Since 2003, the U.S. blood supply has been routinely screened for WNV RNA; as a result, transfusion-associated WNV infection is rare (CDC 2003b). The Food and Drug Administration recommends that blood collection agencies perform WNV nucleic acid amplification test (NAAT) year-round on all blood donations, either in minipools of six or sixteen donations (depending on test specifications) or as individual donations. Organ and tissue donors are not routinely screened for WNV infection (Nett et al. 2012).

Clinical Presentation

An estimated 70-80% of human WNV infections are subclinical or asymptomatic (Mostashari et al. 2001, Zou et al. 2010). Most symptomatic persons experience an acute systemic febrile illness that often includes headache, myalgia, or arthralgia; gastrointestinal symptoms and a transient maculopapular rash also are commonly reported (Watson et al. 2004, Hayes et al. 2005b, Zou et al. 2010). Less than 1% of infected persons develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis (Hayes et al. 2005b). WNV meningitis is clinically indistinguishable from aseptic meningitis due to most other viruses (Sejvar and Marfin 2006). Patients with WNV encephalitis usually present with seizures, mental status changes, focal neurologic deficits, or movement disorders (Sejvar and Marfin 2006). WNV acute flaccid paralysis is often clinically and pathologically identical to poliovirus-associated poliomyelitis, with damage of anterior horn cells, and may progress to respiratory paralysis requiring mechanical ventilation (Sejvar and Marfin 2006). WNV-associated Guillain-Barré syndrome has also been reported and can be distinguished from WNV poliomyelitis by clinical manifestations and electrophysiologic testing (Sejvar and Marfin 2006). Cardiac dysrhythmias, myocarditis, rhabdomyolysis, optic neuritis, uveitis, chorioretinitis, orchitis, pancreatitis, and hepatitis have been described rarely with WNV infection (Hayes et al. 2005b).

Clinical Evaluation and Diagnosis

WNV disease should be considered in the differential diagnosis of febrile or acute neurologic illnesses associated with recent exposure to mosquitoes, blood transfusion or organ transplantation, and of illnesses in neonates whose mothers were infected with WNV during pregnancy or while breastfeeding. In addition to other more common causes of encephalitis and aseptic meningitis (e.g., herpes simplex virus and enteroviruses), other arboviruses (e.g., La Crosse, St. Louis encephalitis, Eastern equine encephalitis, Western equine encephalitis and Powassan viruses) should also be considered in the differential etiology of suspected WNV illness.

WNV infections are most frequently confirmed by detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or cerebrospinal fluid (CSF). The presence of anti-WNV IgM is usually good evidence of recent WNV infection, but may indicate infection with another closely related flavivirus (e.g., St. Louis encephalitis). Because anti-WNV IgM can persist in some patients for >1 year, a positive test result occasionally may reflect past infection unrelated to current disease manifestations. Serum collected within 8 days of illness onset may lack detectable IgM, and the test should be repeated on a convalescent-phase sample. IgG antibody generally is detectable shortly after the appearance of IgM and persists for years. Plaque-reduction neutralization tests (PRNT) can be performed to measure specific virus-neutralizing antibodies. A fourfold or greater rise in neutralizing antibody titer between acute- and convalescent-phase serum specimens collected 2 to 3 weeks apart may be used to confirm recent WNV infection and to discriminate between cross-reacting antibodies from closely related flaviviruses.

Viral culture and WNV NAAT can be performed on acute-phase serum, CSF, or tissue specimens. However, by the time most immunocompetent patients present with clinical symptoms, WNV RNA may not be detectable, thus negative results should not be used to rule out an infection. NAAT may have utility in certain clinical settings as an adjunct to detection of IgM. Among patients with West Nile fever, combining NAAT and IgM detection identified more cases than either procedure alone (Tilley et al. 2006). NAAT may prove useful in immunocompromised patients, when antibody development is delayed or absent. Immunohistochemical staining can detect WNV antigens in fixed tissue, but negative results are not definitive. See the Laboratory Diagnosis and Testing chapter for additional information.

Passive Surveillance and Case Investigation

WNV disease is a nationally-notifiable condition and is reportable in most, if not all, states and territories. Most disease cases are reported to public health authorities from public health or commercial laboratories; healthcare providers also submit reports of suspected cases. State and local health departments are responsible for ensuring that reported human disease cases meet the national case definitions. The most recent case definitions for confirmed and probable neuroinvasive and non-neuroinvasive domestic arboviral diseases were approved by the CSTE in 2011 (Appendix 1). Presumptive WNV-viremic donors are identified through universal screening of the blood supply; case definitions and reporting practices for viremic donors vary by jurisdiction and blood services agency.

All identified WNV disease cases and presumptive viremic blood donors should be investigated promptly. Jurisdictions may choose to interview the patient's health care provider, the patient, or both depending on information needs and resources. Whenever possible, the following information should be gathered:

- Basic demographic information (age, sex, race/ethnicity, state and county of residence)

- Clinical syndrome (e.g., asymptomatic blood donor, uncomplicated fever, meningitis, encephalitis, acute flaccid paralysis)
- Illness onset date and/or date of blood donation
- If the patient was hospitalized and if he/she survived or died
- Travel history in the four weeks prior to onset
- If the patient was an organ donor or a transplant recipient in the 4 weeks prior to onset
- If the patient was a blood donor or blood transfusion recipient in the 4 weeks prior to onset
- If the patient was pregnant at illness onset
- If the patient is an infant, was he/she breastfed before illness onset

If the patient donated blood, tissues or organs in the four weeks prior to illness onset, immediately inform the blood or tissue bank and public health authorities. Similarly, any WNV infections temporally-associated with blood transfusion or organ transplantation should be reported. Prompt reporting of these cases will facilitate the identification and quarantine of any remaining infected products and the identification of any other exposed recipients so they may be managed appropriately.

Passive surveillance systems are dependent on clinicians considering the diagnosis of an arboviral disease and obtaining the appropriate diagnostic test, and reporting of laboratory-confirmed cases to public health authorities. Because of incomplete diagnosis and reporting, the incidence of WNV disease is underestimated. Reported neuroinvasive disease cases are considered the most accurate indicator of WNV activity in humans because of the substantial associated morbidity. In contrast, reported cases of non-neuroinvasive disease are more likely to be affected by disease awareness and healthcare-seeking behavior in different communities and by the availability and specificity of laboratory tests performed. Surveillance data for non-neuroinvasive disease should be interpreted with caution and generally should not be used to make comparisons between geographic areas or over time.

Enhanced Surveillance Activities

Enhanced surveillance for human disease cases should be considered, particularly when environmental or human surveillance suggests that an outbreak is suspected or anticipated. Educating healthcare providers and infection control nurses about the need for arbovirus testing, and reporting of all suspected cases could increase the sensitivity of the surveillance system. This may be accomplished through distribution of print materials, participation in local hospital meetings and grand rounds, and providing lectures/seminars. Public health agencies should also work to establish guidelines and protocols with local blood collection agencies for reporting WNV viremic blood donors.

At the end of the year, an active review of medical records and laboratory results from local hospitals and associated commercial laboratories should be conducted to identify any previously unreported cases. In addition, an active review of appropriate records from blood collection agencies should be conducted to identify any positive donors that were not reported.

Environmental Surveillance

Mosquito-Based WNV Surveillance

Mosquito-based surveillance consists of the systematic collection of mosquito samples and screening them for arboviruses. Mosquitoes become infected with WNV primarily through taking blood meals from infected birds. However, WNV may be passed from infected female mosquitoes to their eggs, resulting in infected offspring (i.e., vertical transmission) (Anderson et al. 2006). Vertical transmission is likely responsible for virus maintenance over the winter in northern parts of the country, but the extent of its contribution to virus amplification and human risk during the peak transmission season is not well understood.

The principal enzootic and epidemic vectors vary regionally across the United States. In the northern states, *Culex pipiens* mosquitoes are the primary vectors (Savage et al 2007) with *Cx. salinarius* serving as an important vector in portions of the Northeast (Anderson et al. 2004, 2012, Molaei et al. 2006). *Culex quinquefasciatus* is the main vector in the southern states, and *Culex tarsalis* is an important vector in western states where it overlaps the distribution of *Cx. pipiens* and *Cx. quinquefasciatus* and likely enhances transmission in these areas (Reisen et al. 2005, Andreadis 2012). Therefore, mosquito-based surveillance programs for WNV in the United States primarily target these *Culex* species and may include other species suspected of contributing to transmission and human risk in local areas.

Mosquito-based surveillance is an integral component of an integrated vector management program and is the primary tool for quantifying WNV virus transmission and human risk (Moore et al. 1993). The principal functions of a mosquito-based surveillance program are to:

- Collect data on mosquito population abundance and virus infection rates in those populations.
- Provide indicators of the threat of human infection and disease and identify geographic areas of high-risk.
- Support decisions regarding the need for and timing of intervention activities (i.e., enhanced vector control efforts and public education programs).
- Monitor the effectiveness of vector control efforts.

Mosquito-based WNV monitoring has several positive attributes that contribute to its value in surveillance programs. These include:

- Quick turn-around of results. Mosquito samples can be processed quickly, usually within a few days. Some programs maintain local, in-house laboratories where samples are processed daily leading to rapid results.
- Collecting adult mosquitoes provides information about vector species community composition, relative abundance, and infection rates. This provides the data needed for rapid computation of infection indices and timely risk assessment.
- Maintaining consistent programs over the long-term provides a baseline of historical data that can be used to evaluate risk and guide control operations.

However, there are some limitations to mosquito-based surveillance. Virus may not be detected in the mosquito population if the infection rates are very low (i.e., early in the transmission season) or if only small sample sizes are tested. In addition, WNV transmission ecology varies regionally, and surveillance practices vary among programs (e.g., number and type of traps, testing procedures), which limits the

degree to which surveillance data can be compared across regions. This prohibits setting national thresholds for assessing risk and implementing interventions. Developing useful thresholds requires consistent effort across seasons to assure the surveillance indices and their association to human risk is comparable over time, and may require mosquito surveillance and human disease incidence data from several transmission seasons.

Specimen Collection and Types of Traps

Adult mosquitoes are collected using a variety of trapping techniques. Adequate sampling requires regular (weekly) trapping at fixed sites throughout the community that are representative of the habitat types present in the area. The commonly used types of mosquito traps for WNV surveillance sample host-seeking mosquitoes or gravid mosquitoes (carrying eggs) seeking a place to lay eggs (oviposition site).

Traps used to sample host-seeking mosquitoes are available in several configurations. The most commonly used are based on the CDC miniature light trap (Sudia and Chamberlain 1962). The CDC miniature light trap and similar configurations are lightweight and use batteries to provide power to a light source and fan motor. CO_2 (usually dry ice) is frequently used as an additional attractant. In some programs, the light sources are removed to minimize the capture of other nocturnal insects that are attracted to light, such as moths and beetles. In those cases CO_2 is the only attractant used. The advantage of light traps is that they collect a wide range of mosquito species (McCardle et al. 2004), which provides information about both primary and secondary vectors and a better understanding of the species composition in an area. A limitation of light traps is that the collections in certain locations and times may consist largely of unfed, nulliparous individuals (McCardle et al. 2004), which greatly reduces the likelihood of detecting WNV and other arboviruses. Also, not all mosquito species are attracted to light traps (Miller et al. 1969) and the numbers captured may not reflect the population size of a particular species (Bidlingmayer 1967). Light traps are of little use in sampling day-active time mosquitoes such as *Ae. albopictus* (Haufe and Burgess 1960, Unlu and Farajollah 2012), though these species can be collected in other traps such as the BG Sentinel (Krokel et al. 2006). However, the role of these species in WNV transmission is not well understood. The three major WNV vectors (*Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. tarsalis)* can be collected in light traps, and some surveillance programs rely on light traps alone. This should be done with the understanding that, while effective in collecting large numbers of *Cx. tarsalis*, light traps typically collect relatively few *Cx. pipiens* or *Cx. quinquefasciatus* and the resulting small sample sizes may reduce the ability to accurately estimate WNV infection rates. Gravid traps are more effective in collecting *Cx. pipiens* and *Cx. quinquefasciatus* in urban areas (Andreadis and Armstrong 2007, Reisen et al. 1999).

Gravid traps target gravid females (i.e., those carrying mature eggs) of the *Cx. pipiens* complex (Reiter et al. 1986). The strength of gravid traps is that gravid females have previously taken a blood meal, which greatly increases the likelihood of capturing WNV infected individuals and thus detecting virus. Gravid traps can be baited attractants such as fresh or dry grass clippings infusions, rabbit chow infusions, cow manure, fish oil, or other materials that mimic the stagnant water in habitats where these species lay eggs. The different infusions vary in attractiveness (Burkett et al 2004, Lampman et al. 1996). It is advisable that infusion preparations are consistent within a surveillance program because variations may lead to changes in number and/or type of species captured. One limitation of gravid traps is that they selectively capture mosquitoes in the *Cx. pipiens* complex, and therefore provide limited information on species composition within a region (Reiter et al. 1986).

Several other traps may be used to collect mosquitoes for WNV monitoring. Collecting resting mosquitoes provides a good representation of vector population structure since un-fed, gravid and blood-fed females (as well as males) may be collected (Service 1992). Since resting populations typically provide samples that are representative of the population they can also provide more representative WNV infection rates. Resting mosquitoes can be collected using suction traps such as the CDC resting trap (Panella et al. 2011), and by using handheld or backpack mechanical aspirators (Nasci 1981) to remove mosquitoes from natural resting harborage or artificial resting structures (e.g., wooden resting boxes, red boxes, fiber pots and other similar containers) (Service 1992). Because of the wide variety of resting sites and the low density of resting mosquitoes in most locations, sampling resting populations is labor intensive and sufficient sample sizes are often difficult to obtain.

Host-baited traps, usually employing chickens or pigeons as bait, can collect mosquito vectors of interest in large numbers. However, these methods require use of live animals and adherence to animal use requirements and permitting. In addition, they are similar to light traps in that collections in certain locations and times may consist largely of unfed nulliparous individuals. Human landing collections have been used to accurately measure the population of human-feeding mosquitoes in an area and can be quite valuable in monitoring the effectiveness of adult mosquito control efforts. However, human landing collections may expose collectors to infected mosquitoes and is not recommended as a sampling procedure in areas where WNV transmission is occurring.

Specimen Handling and Processing

Since mosquito-based WNV surveillance relies on identifying WNV in the collected mosquitoes through detection of viral proteins, viral RNA, or live virus (see Diagnostics section), efforts should be made to handle and process the specimens in a way that minimizes exposure to conditions (e.g., heat, successive freeze-thaw cycles) that would degrade the virus. Optimally, a cold chain should be maintained from the time mosquitoes are removed from the traps to the time they are delivered to the processing laboratory, and through any short-term storage and processing. Transport mosquitoes from the field in a cooler either with cold packs or on dry ice. Sort and identify the mosquitoes to species on a chill-table if available. This is particularly important if the specimens will be tested for infectious virus or viral antigen. However, lack of a cold chain does not appear to reduce the ability to detect WNV RNA by RT-PCR (Turell et al. 2002).

Mosquitoes are generally tested in pools of 50 to 100 specimens grouped by species, date, and location of collection. Larger pool sizes may result in a loss of sensitivity (Sutherland and Nasci 2007). Follow package instructions that may indicate smaller pool sizes if using a commercial WNV assay – see Diagnostics section. Usually only female mosquitoes are tested in routine WNV surveillance programs. If WNV screening is not done immediately after mosquito identification and pooling, the pooled samples should be stored frozen, optimally at -70^{O}C, but temperatures below freezing may suffice for short-term storage.