

Centers for Disease Control and Prevention

**Epidemic/Epizootic West Nile Virus in  
the United States:  
Guidelines for Surveillance, Prevention,  
and Control**

**U.S. Department of Health and Human Services  
Public Health Service  
Centers for Disease Control and Prevention  
National Center for Infectious Diseases  
Division of Vector-Borne Infectious Diseases  
Fort Collins, Colorado  
3<sup>rd</sup> Revision**

2003



## Table of Contents

Introduction .....	5
I. SURVEILLANCE .....	7
A. Ecologic Surveillance.....	7
1. Avian .....	7
2. Equine .....	13
3. Mosquito.....	15
B. Surveillance for Human Cases.....	18
1. Recent Experience .....	18
2. Types of Surveillance .....	20
3. Specimens.....	21
4. Surveillance Case Definition .....	22
5. Minimal Components of a Human Surveillance System .....	22
C. Geography and Timing.....	22
1. Northeastern and Midwestern U.S. ....	22
2. Southern U.S.....	22
3. Western U.S. ....	22
4. Other Areas of the Western Hemisphere .....	22
II. LABORATORY DIAGNOSIS.....	24
A. Biocontainment.....	24
1. Laboratory Safety Issues.....	24
2. Shipping of Agents .....	25
B. Serologic Laboratory Diagnosis .....	25
1. Human.....	25
2. Animal .....	26
C. Virologic Laboratory Diagnosis .....	26
1. Virus Isolation.....	26
2. Virus Detection in Tissues.....	27
D. Training and Infrastructure .....	27
1. State and Local Arbovirus Laboratories .....	27
2. Training Programs .....	27
III. PREVENTION AND CONTROL.....	28
A. Surveillance.....	28
1. Larval Mosquito Surveillance .....	28
2. Adult Mosquito Surveillance .....	28
3. Virus Surveillance.....	29
B. Source Reduction.....	29
1. Sanitation .....	30
2. Water Management.....	30

C. Chemical Control.....	31
1. Larviciding .....	31
2. Adulticiding .....	32
D. Resistance Management.....	33
E. Biological Control.....	34
F. Continuing Education of Mosquito Control Workers .....	34
G. Vector Management in Public Health Emergencies .....	34
H. Adult Mosquito Control Recommendations .....	35
I. Determining the Scope of Mosquito Adulticiding Operations .....	36
J. Evaluation of Adult Mosquito Control .....	37
K. Health Education, Public Information, and Human Behavior Change .....	37
1. Key WNV Prevention Messages .....	37
2. Selected Best Practices .....	38
3. Research and Program Development Priorities .....	39
4. Resources .....	40
L. Legislation .....	41
M. Guidelines for a Phased Response to WN Virus Surveillance Data .....	41
IV. HEALTH DEPARTMENT INFRASTRUCTURE .....	45
A. Staffing and Personnel .....	45
B. Training and Consultation .....	45
C. Laboratory Capacity .....	45
1. Testing for West Nile Virus Infections .....	45
D. Developing Local Public Health Agency Infrastructure .....	46
V. INTERJURISDICTIONAL DATA SHARING AND NATIONAL REPORTING OF HUMAN CASES .....	48
A. Human Epidemiological, Clinical, and Laboratory Data Collection .....	48
B. National Reporting of Human WNV Disease Cases .....	48
1. WNME .....	48
2. WNF .....	48
C. Ecologic Data .....	48
1. Accurate Taxonomic Identification of Specimens.....	49
2. Unique Identifier (UID) Numbering System for Specimens .....	49
3. Durable Tagging System for Field-Collected Specimens .....	49
VI. RESEARCH PRIORITIES .....	50
A. Current and Future Geographic Distribution of WNV .....	50
B. Bird Migration as a Mechanism of WNV Dispersal.....	50
C. Vector and Vertebrate Host Relationships and Range.....	50
D. Virus Persistence Mechanisms .....	50
E. Mosquito Biology, Behavior, Vector Competence, Surveillance, and Control.....	50
F. Development and Evaluation of Prevention Strategies .....	50

G. Laboratory Diagnosis .....	51
H. Clinical Spectrum of Disease and Long-Term Prognosis in Humans .....	51
I. Risk Factor Studies .....	51
J. Detailed Clinical Descriptions and Outcome in Human Cases.....	51
K. Viral Pathogenesis .....	51
L. Genetic Relationships and Molecular Basis of Virulence .....	51
M. Vaccine Development for Animals and Humans .....	52
N. Antiviral Therapy for West Nile Virus and Other Flaviviruses.....	52
O. The Economic Cost of WNV Epidemic/Epizootic .....	52
P. WNV Impact on Wildlife.....	52
Q. Investigate Alternate Modes of WNV Transmission to Humans .....	52
Appendix A – National West Nile Virus Surveillance System .....	53
Appendix B – Surveillance Case Definition for West Nile Virus Infection in Equines.....	66
Appendix C – National Surveillance Case Definition for Arboviral Encephalitis/Meningitis .....	68
Appendix D – CDC-Recommended Surveillance Case Definition for WN Fever .....	70
Appendix E – Recommended Framework for Standardized "Extended" Clinical Variables in Studies of Human WNV Disease .....	72
References.....	73

Department of Health and Human Services  
Centers for Disease Control and Prevention  
Julie Louise Gerberding, M.D., M.P.H., Director

National Center for Infectious Diseases (NCID)  
James M. Hughes, M.D., Director  
Stephen M. Ostroff, M.D., Deputy Director

The following CDC, Division of Vector-Borne Infectious Diseases staff members prepared this report:

Duane J. Gubler, Sc.D., Director  
Lyle R. Petersen, M.D., M.P.H., Deputy Director  
John T. Roehrig, Ph.D.  
Grant L. Campbell, M.D., Ph.D.  
Nicholas Komar, Sc.D.  
Roger S. Nasci, Ph.D.  
Emily Zielinski-Gutierrez, Ph.D.  
Anthony A. Marfin, M.D., M.P.H.  
Robert S. Lanciotti, Ph.D.  
Michel L. Bunning, DVM  
Daniel R. O’Leary, DVM  
Mel Fernandez, Deputy Director  
Lauren Dieterich

Barbara B. Tuttle  
Rebecca L. Deavours

Prepared in consultation with:

Association of Public Health Laboratories  
Council of State and Territorial Epidemiologists  
Environmental Protection Agency  
Department of Army  
National Association of County and City Health Officials  
National Institutes of Health  
National Parks Service  
State Public Health Veterinarians  
State Public Health Vector Control Conference  
United States Department of Agriculture, Veterinary Services

## **INTRODUCTION**

In late summer 1999, the first domestically acquired human cases of West Nile (WN) encephalitis were documented in the U.S.<sup>1-6</sup> The discovery of virus-infected, overwintering mosquitoes during the winter of 1999-2000 presaged renewed virus activity for the following spring and precipitated early season vector control and disease surveillance in New York City (NYC) and the surrounding areas.<sup>7,8</sup> These surveillance efforts were focused on identifying and documenting WN virus (WNV) infections in birds, mosquitoes and equines as sentinel animals that could alert health officials to the occurrence of human disease. Surveillance tracked the spread of WNV throughout much of the U.S. between 2000 and 2002. By the end of 2002, WNV activity had been identified in 44 states and the District of Columbia. The 2002 WNV epidemic and epizootic resulted in reports of 4,156 reported human cases of WN disease (including 2,942 meningoencephalitis cases and 284 deaths), 16,741 dead birds, 6,604 infected mosquito pools, and 14,571 equine cases. The 2002 WNV epidemic was the largest recognized arboviral meningoencephalitis epidemic in the Western Hemisphere and the largest WN meningoencephalitis epidemic ever recorded. Significant human disease activity was recorded in Canada for the first time, and WNV activity was also documented in the Caribbean basin and Mexico. In 2002, 4 novel routes of WNV transmission to humans were documented for the first time: 1) blood transfusion, 2) organ transplantation, 3) transplacental transfer, and 4) breast-feeding.

WNV is a member of the family Flaviviridae (genus Flavivirus). Serologically, it is a member of the Japanese encephalitis virus antigenic complex, which includes St. Louis, Japanese, Kunjin, and Murray Valley encephalitis viruses.<sup>9,10</sup> WNV was first isolated in the WN province of Uganda in 1937.<sup>11,12</sup> Human and equine outbreaks have been recorded in portions of Africa, southern Europe, North America, and Asia.<sup>13,14</sup>

Although it is still not known when or how WNV was introduced into North America, international travel of infected persons to New York, importation of infected birds or mosquitoes, or migration of infected birds are all possibilities. In humans, WNV infection usually produces either asymptomatic infection or mild febrile disease, sometimes accompanied by rash, but it can cause severe and even fatal diseases in a small percentage of patients. The human case-fatality rate in the U.S. has been 7% overall, and among patients with neuroinvasive WNV disease, 10%.

Unlike WNV within its historical geographic range, or St. Louis encephalitis (SLE) virus in the Western Hemisphere, mortality in a wide variety of bird species has been a hallmark of WNV activity in the U.S. The reasons for this are not known; however, public health officials have been able to use bird mortality (particularly birds from the family Corvidae) to effectively track the movement of WNV. WNV has now been shown to affect 162 species of birds. Previous early-season field studies have determined that areas with bird mortality due to WNV infection were experiencing ongoing enzootic transmission. However, most birds survive WNV infection as indicated by the high seroprevalence in numerous species of resident birds within the regions of most intensive virus transmission. The contribution of migrating birds to natural transmission cycles and dispersal of both WN and SLE viruses is poorly understood.

WNV has been transmitted principally by *Culex* species mosquitoes, the usual vectors of SLE virus. Thirty-six species of mosquitoes have been shown to be infected with WNV. This wide variety of WNV-infected mosquito species has widened this virus' host-range in the U.S.: 27 mammalian species have been shown to be susceptible to WNV infection and disease has

been reported in 20 of these (including humans and horses). It must be remembered, however, that the detection of WNV in a mosquito species is necessary but not sufficient to implicate that species as a competent vector of WNV.

Since 1999, the Centers for Disease Control and Prevention (CDC) and a variety of other U.S. governmental agencies and partners have sponsored yearly national meetings of arbovirologists, epidemiologists, laboratorians, ecologists, vector-control specialists, wildlife biologists, communication experts, and state and local health and agriculture officials to assess the implications of the WNV introduction into the U.S. and to refine the comprehensive national response plan. Recommendations from these meetings have been used to develop and to update these guidelines.<sup>15,16</sup> This document is available electronically from the CDC Web site at: <http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm>.

To assist guideline implementation in 2000, CDC developed an electronic-based surveillance and reporting system (ArboNet) to track WNV activity in humans, horses, other mammals, birds and mosquitoes. In 2003, the ArboNet surveillance system has been updated to streamline reporting to CDC of WNV activity by the state public health departments.

Today's rapid transport of people, animals, and commodities increase the likelihood that other introductions of exotic pathogens will occur. CDC continues to implement its plan titled "Preventing Emerging Infectious Diseases, a Plan for the 21<sup>st</sup> Century".<sup>17</sup>



## I. SURVEILLANCE

A universally applicable arbovirus surveillance system does not exist. In any given jurisdiction, surveillance systems should be tailored according to the probability of arbovirus activity and available resources. In jurisdictions without pre-existing vector-borne disease surveillance and control programs, newly developed avian-based and/or mosquito-based arbovirus surveillance systems will be required. In some, resurrection of previously abandoned systems will be necessary. In others, modification and/or strengthening of existing arbovirus surveillance systems (i.e., those intended to monitor eastern equine encephalitis [EEE], western equine encephalitis [WEE], and/or St. Louis encephalitis [SLE] virus activity) will be the most appropriate response. In yet other jurisdictions in which the probability of arbovirus activity is very low and/or resources to support avian-based and/or mosquito-based surveillance are unavailable, laboratory-based surveillance for neurologic disease in humans and equines should be employed at minimum.

Seasonality of surveillance activities may vary depending upon geographic region. With the anticipated spread of West Nile virus (WNV) to all of the 48 contiguous United States in 2003, all states should initiate surveillance after mosquitoes become active in the spring.

Appropriate and timely response to surveillance data is the key to preventing human and animal disease associated with WNV and other arboviruses. That response must include effective mosquito control and public education without delay, if an increasing intensity of virus activity is detected by bird- or mosquito-based surveillance systems (see Section III.M). For basic information on arbovirus surveillance, see CDC Guidelines for Arbovirus Surveillance Programs in the United States,<sup>18</sup> this document can be obtained from CDC's Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado, and is also available from the CDC Web site at: <http://www.cdc.gov/ncidod/dvbid/arbor/arboguid.htm>.

### A. *Ecologic Surveillance*

Detection of WNV in bird and mosquito populations helps health officials predict and prevent human and domestic animal infections. Surveillance to detect WNV should focus on the avian and mosquito components of the enzootic transmission cycle. Non-human mammals, particularly equines, may also serve as effective sentinels because a high intensity of mosquito exposure makes them more likely to be infected than people. Descriptions of the avian-, mosquito-, and non human mammal-based surveillance strategies follow.

#### 1. **Avian**

##### a) Avian morbidity/mortality surveillance

Avian morbidity/mortality surveillance appears to be the most sensitive early detection system for WNV activity, and should be a component of every state's arbovirus surveillance program. Its utility for monitoring ongoing transmission in a standardized fashion is currently being investigated, but should include at least two basic elements: the timely reporting and analysis of dead bird sightings and the submission of selected individual birds for WNV testing.

GOAL OF AVIAN MORBIDITY/MORTALITY SURVEILLANCE: Utilize bird mortality associated with WNV infection as a means of detecting WNV activity in a location.

1) *Protocols and specimens*

The level of effort involved in this surveillance activity will depend on a risk assessment in each jurisdiction. Generally, avian surveillance should be initiated when local adult mosquito activity begins in the spring. A database should be established to record and analyze dead bird sightings with the following suggested data: caller identification and call-back number, date observed, location geocoded to the highest feasible resolution, species, and condition. Samples from birds in good condition (unscavenged and without obvious decomposition or maggot infestation) may be submitted for laboratory testing. As with all dead animals, carcasses should be handled carefully, avoiding direct contact with skin. For greatest sensitivity, a variety of bird species should be tested, but corvids should be emphasized.<sup>19</sup> The number of bird specimens tested will be dependent upon resources and whether WNV-infected birds have been found in the area; triage of specimens may be necessary on the basis of sensitive species (such as corvids) and geographic location. Many jurisdictions may limit (or even stop) avian mortality surveillance once WNV is confirmed in their region. It is suggested that avian mortality surveillance be continued in each region as long as it remains necessary to know whether local transmission persists, because dead-bird-based surveillance is the most sensitive method for detection of WNV activity in most regions.

A single organ specimen from each bird is sufficient to detect WNV or viral RNA. Kidneys, brains, or hearts are preferable.<sup>20-22</sup> Oral swabs from corvids have been validated as a sensitive alternative to organ samples, and because fewer resources are necessary to acquire them, oral swabs are the preferred specimen from corvid carcasses.<sup>23</sup> Testing involves isolation of infectious virus, specific RNA detection by reverse transcription-polymerase chain reaction (RT-PCR),<sup>24</sup> or antigen detection,<sup>25,26</sup> and will generally be positive within 1-2 weeks after specimen submission.

2) *Recent experience*

Analysis of recent avian morbidity and mortality data indicated that

- (a) The American crow was the most sensitive species for avian morbidity/mortality surveillance in northern regions. However, some areas did not have WNV-positive American crows, but only WNV-positive birds of other species. In southern regions, blue jays have been more sensitive than crows.
- (b) Almost all of the positive birds were found singly and not as part of a mass die-off at a single time and place.
- (c) Approximately one-third of the WNV-positive birds had signs of trauma on necropsy.

- (d) Many WNV-positive birds did not have pathology indicative of WNV infection on necropsy. No lesions are pathognomonic for WNV infection.
- (e) WNV-positive dead birds usually provided the earliest indication of viral activity in an area. In 2002, the detection of WNV-infected dead birds was the first positive surveillance event in 1,534 (61%) of 2,531 counties reporting WNV activity.
- (f) The detection of WNV-positive dead birds preceded reports of human cases (although knowledge of the test result did not necessarily predate the onset of human cases). In 2002, 527 (89%) of 589 counties reporting human WN meningoencephalitis cases first detected WNV transmission in animals. In 327 (72%) of these 527 counties, detection of WNV-infected dead birds was the first positive surveillance event, preceding human illness onset by a median of 38.5 days (range, 2-252 days).
- (g) Many counties with human cases of WNV infection tended to have high dead bird surveillance indices, both WNV-positive and sightings. Notable exceptions included sparsely populated counties, particularly those in the midwestern states.<sup>27,28</sup>
- (h) Experimental evidence of direct transmission among corvids and gulls exists, but whether this occurs in nature is unknown.<sup>29</sup> If it does, then in some settings, virus-infected mosquitoes might not be necessary to maintain enzootic transmission cycles.

3) *Advantages of avian morbidity/mortality surveillance include the following:*

- (a) Certain species of birds, in particular corvids (e.g., crows and jays) experience high clinical attack rates.
- (b) The size and coloration of certain dead birds makes them conspicuous (e.g., crows).
- (c) RT-PCR and antigen-detection assays can be used to rapidly detect WN viral RNA and protein, respectively, in tissues, even if the tissue is partly decomposed. Both assays have now been adapted for field applications.
- (d) Due to public involvement in reporting dead bird sightings, dead wild birds are readily available over a much wider region than can be sampled by other surveillance methods.
- (e) Detection of WNV in dead birds likely signifies local transmission.<sup>30</sup>
- (f) This type of surveillance provides a temporally and spatially sensitive method for the detection of WNV activity.
- (g) It can be used for early detection and possibly also for ongoing monitoring of WNV transmission.
- (h) It may be used to estimate risk of human infection with WNV.<sup>27,31,32</sup>

4) *Disadvantages of avian morbidity/mortality surveillance include the following:*

- (a) Dead bird surveillance data from different jurisdictions are difficult to compare.
- (b) Birds are highly mobile and often have extensive home ranges, so that the site of death may be distant from the site of infection (especially after the breeding season, when birds are generally less territorial).
- (c) Collection, handling, shipping, and processing of birds or their clinical specimens is cumbersome.

- (d) Systems for handling, processing, and testing have at times been overwhelmed by high public response and public expectations.
- (e) The long-term usefulness of this system is uncertain because natural selection for disease-resistant birds may occur, populations of susceptible species may become very low, or the virus may evolve, resulting in low or no avian mortality. In areas where WNV annually recurs, intense environmental sampling might not be as useful.
- (f) Success is influenced by public participation, which is highly variable, and depends on the number of public outreach programs, level of public concern, etc.
- (g) The system may be less sensitive in rural areas, where there are fewer persons to observe dead birds over a wider geographic area. In the western U.S., low observer density is coupled with the presence of a vector (*Culex tarsalis*) that is less ornithophilic, resulting in fewer reports of dead birds relative to other non-avian surveillance indicators.

b) Live bird surveillance

Live-bird surveillance has been used traditionally both to detect and monitor arbovirus transmission (e.g., for SLE, EEE and WEE viruses). Two approaches are captive sentinel surveillance (typically using chickens, but other species have been used as well), and free-ranging bird surveillance.<sup>33</sup> Both depend on serological testing, which generally requires at least 3 weeks to detect and confirm an infection. Successful application of these approaches requires extensive knowledge of local transmission dynamics. It is recommended that further research be done before relying on sentinel birds as a primary means of WNV surveillance. Use of sentinel birds may require institutional animal use and care protocols, and other authorization permits.

GOAL OF LIVE-BIRD SURVEILLANCE: Utilize seroconversions in captive or free-ranging bird species as indicators of local WNV activity.

1) *Captive sentinel surveillance*

Although an ideal captive avian sentinel for WNV -- or any other arbovirus -- may not exist, such a species would meet the following criteria: 1) is universally susceptible to infection, 2) has a 100% survival rate from infection and universally develops easily detectable antibodies, 3) poses no risk of infection to handlers, and 4) never develops viremia sufficient to infect vector mosquitoes.<sup>18</sup> Captive sentinels have been effectively used to monitor transmission of arboviruses in a standardized fashion, including SLE virus in California and Florida, especially in historical enzootic transmission foci. Captive sentinel flocks should be placed in likely transmission foci (e.g., near vector breeding sites or adult mosquito congregation sites), and presented appropriately to allow feeding by enzootic WNV vectors. Alternatively, pre-existing captive birds (e.g., domestic poultry or pigeons, or zoo birds) may be used as sentinels.

- (a) Protocols and specimens  
Whole blood can be collected and centrifuged for serum. Serum is screened by either hemagglutination inhibition (HI), enzyme-linked immunosorbent assay (ELISA) or plaque-reduction neutralization test (PRNT).<sup>34</sup> It is important to note that the extraction of avian serum samples to remove non specific inhibitors of hemagglutination for use in the HI test follows procedures different from those used in tests of human serum samples.<sup>35</sup> Positive tests must be confirmed by neutralization to rule out false positives and cross-reactions due to infection with related flaviviruses (e.g., SLE virus).
  
- (b) Recent experience
  - (i) In 2000, sentinel chickens were used in selected counties in New York State, New York City (NYC), New Jersey, Pennsylvania, Maryland, and Delaware. Small numbers of seroconversions were detected late in the season in New Jersey and New York. As used in 2000, chickens were ineffective sentinels. In NYC in 2001, sentinel chickens were placed in known transmission foci and seroconverted earlier in the season, but not earlier than the first human cases. In 2002, hundreds of sentinel chickens in the Southeast seroconverted, but these were rarely the earliest indicators of WNV activity at the county level.
  - (ii) IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) testing of experimentally infected chickens points to the need for biweekly sampling of sentinels.<sup>36</sup>
  - (iii) Experimental studies have shown that chickens, pigeons, and pheasants (CDC, unpublished data) are candidate sentinels due to their susceptibility to infection, low mortality, and relative incompetence as amplifying hosts. However, small amounts of WNV were detected in cloacal swabs from infected chickens and pigeons.<sup>29,37</sup>
  - (iv) Field studies of avian seroprevalence in Queens in 1999 indicated that captive chickens frequently were infected.<sup>38</sup> In Staten Island in 2000, captive pigeons frequently were infected.<sup>39</sup>
  - (v) Some mortality in chickens was attributed to WNV at various locations in New York State.<sup>40</sup>
  
- (c) Advantages of sentinel captive bird surveillance include the following:
  - (i) Chickens have been successfully used in flavivirus surveillance for over 6 decades.
  - (ii) Birds are readily fed upon by *Culex* mosquitoes.
  - (iii) Captive birds can be serially bled, making the geographic location of infection definite.
  - (iv) The system is flexible and therefore can be expanded and contracted as appropriate.
  - (v) Mosquito-abatement districts can maintain and bleed flocks and submit specimens for testing.

- (vi) Collection of specimens is inexpensive compared with the costs of free-ranging bird surveillance.
- (d) Disadvantages of captive sentinel surveillance include the following:
  - (i) Sentinel flocks detect only focal transmission, requiring multiple flocks be positioned in representative geographic areas. This is particularly true when vector mosquitoes have short flight ranges (e.g., *Culex pipiens*).
  - (ii) Flocks are subject to vandalism and theft.
  - (iii) Flocks must be protected from predators.
  - (iv) Flock set-up and maintenance (i.e., birds, cages, feed, transportation) are expensive. Training is required for proper maintenance and sampling.
  - (v) Pre-existing flocks may already have been exposed due to previous local WNV transmission.

## 2) *Free-ranging bird surveillance*

Free-ranging birds provide the opportunity for sampling important reservoir host species and may be used both for early detection and for monitoring virus activity. This type of surveillance has been used effectively for SLE, EEE and WEE virus surveillance in several states. In each geographic area, the optimal free-ranging bird species to be monitored should be determined by serosurveys. The best species for serologic surveillance are those in which infection is rarely, if ever, fatal, and population replacement rates are high, ensuring a high proportion of uninfected individuals.

### (a) Protocols and specimens

The use of free-ranging birds requires differentiation of recent infection from infections acquired in previous years. For most species, assays for detection of IgM antibody will not be available and other tests such as IgG (IgY)-detection ELISAs<sup>41,42</sup> and the PRNT<sup>34</sup> must be used to detect WNV-specific antibody. Antibody-positive birds less than 1 year old may be presumed to have been infected recently (during current transmission season). Weak seropositivity in very young birds (less than 1 month old) may be due to maternal transfer of antibody. Seroconversion in older birds is also evidence of recent transmission, but requires frequent recapture for acquisition of multiple specimens from uniquely banded individuals during the course of the transmission season. WNV seropositivity among after-hatch-year birds, when determined from a single serum specimen, should not be interpreted or reported as evidence of recent infection. State and federal permits are required for capture and banding of federally-protected migratory birds.

### (b) Recent experience

- (i) In urban epizootic transmission foci in NYC, several common species (i.e., house sparrows, cardinals, catbirds, mourning doves, rock doves) developed high seroprevalence, making them

strong candidate sentinels, although other species may be important in other locations.<sup>38,39</sup>

- (ii) A comparison of free-ranging bird surveillance in NYC in 2001 found that much greater effort was required for this surveillance system compared with other surveillance systems (Green Street Scientific, LLC, unpublished data). Similar observations have been made in Indiana, Louisiana, New Jersey, Ohio, and Texas.
  
- (c) Advantages of free-ranging bird surveillance include the following:
  - (i) It has a long history of successful use in flavivirus surveillance.
  - (ii) Local movement of resident wild birds may increase contact with enzootic transmission foci, thus increasing sensitivity (relative to captive sentinels).
  - (iii) Set-up or maintenance costs may be minimal.
  - (iv) Its sampling capability is highly flexible.
  - (v) It permits evaluation of herd immunity among important amplifying hosts.
  - (vi) Owner confidentiality may be less of an issue.
  
- (d) Disadvantages of free-ranging bird surveillance include the following:
  - (i) Interpretation of serologic results is complex.
  - (ii) Handling and venipuncture of birds increases the risk of exposure to pathogens in blood and feces.
  - (iii) Movement of free-ranging wild birds makes it impossible to know where an infection was acquired.
  - (iv) Most birds are protected by federal law, and their collection and sampling requires state and federal permits. Banding permits require complex data reporting.
  - (v) Training is required for live-trapping, blood-sampling, handling, and accurate determination of the species and age of wild birds.
  - (vi) It is generally not feasible to serially bleed individual free-ranging birds because of low recapture rates (although banding can be useful).

## 2. Equine

Equines appear to be important sentinels of WNV epizootic activity and human risk, at least in some geographic regions. In addition, equine health is an important economic issue. Therefore, surveillance for equine WNV disease should be conducted in jurisdictions where equines are present. Veterinarians, veterinary service societies/agencies, and state agriculture departments are essential partners in any surveillance activities involving equine WNV disease. A working surveillance case definition of clinical WNV infection in equines is presented in Appendix B.

**GOALS OF EQUINE DISEASE SURVEILLANCE:** To use data on equine WNV disease cases to assess the threat of human disease, identify geographic areas of high risk, and assess the need for and timing of interventions.

### a) Protocols and Specimens

- 1) Serum and cerebrospinal fluid (CSF) for antibody testing. Because an equine WNV vaccine is now in widespread use, a complete vaccination history should accompany all specimens submitted for antibody testing.
- 2) Necropsy tissues (especially brain and spinal cord) for gross pathology, histopathology, RT-PCR, virus isolation, and immunohistochemistry. The differential diagnosis of equine encephalitis includes, but is not limited to, the other arboviral encephalitides and rabies.

b) Recent experience

- 1) In 2002, equine WNV disease cases were the first indication of WNV activity in 95 (16%) of the 589 counties where human disease was reported. The majority of these 95 counties were located in the central and western U.S.
- 2) In general, equine WNV disease cases have been scattered. Few case clusters have been documented.
- 3) In fatal equine WNV disease cases, pathological findings have been non-specific. Pathognomonic lesions have not been described.
- 4) A licensed equine WNV vaccine has been available in the U.S. since 2001. No studies of efficacy have been published.

c) Advantages of equine disease surveillance include the following:

- 1) Equines are highly conspicuous, numerous, and widely distributed in some areas. They may be particularly useful sentinels in rural areas, where dead birds may be less likely to be detected.
- 2) Some equines are routinely bled and tested for other pathogens.
- 3) Ill equines have been one of the earliest, if not the earliest, sentinels of WNV activity in some geographic areas.

d) Disadvantages of equine disease surveillance include the following:

- 1) In some geographic areas, equines may not be an early sentinel (i.e., human WNV disease cases may occur simultaneously with or soon after equine cases).
- 2) Necropsies are expensive and logistically difficult.
- 3) Equines are not present or abundant in many areas of the U.S. (e.g., densely populated metropolitan areas), and proximity of equines to human populations varies.
- 4) Widespread use of equine WNV vaccines may decrease the incidence of equine WNV disease and therefore the usefulness of equines as sentinels.



- 5) Because the costs of clinical equine specimen collection and testing are usually borne directly by the owner, economic factors work against the submission and testing of equine specimens for arboviral infections.

e) Minimal components of an equine surveillance program

- 1) All equine neurologic disease cases should be promptly reported; the equines should be tested for infection with WNV and other arboviruses as geographically appropriate, and for rabies.
- 2) Clusters of equine neurologic disease cases should be promptly investigated.

### 3. Mosquito

While dead-bird-based surveillance has proven to be the most sensitive method of detecting WNV presence in an area, mosquito-based surveillance remains the primary tool for quantifying the intensity of virus transmission in an area, and should be a mainstay in most surveillance programs for WNV and other arboviruses.

GOALS OF MOSQUITO-BASED SURVEILLANCE: To 1) use data on mosquito populations and virus infection rates to assess the threat of human disease; 2) identify geographic areas of high risk; 3) assess the need for and timing of interventions; 4) identify larval habitats for targeted control; 5) monitor the effectiveness of this type of surveillance and improve prevention and control measures; and 6) develop a better understanding of transmission cycles and potential vector species.

a) Protocols and specimens

- 1) Adult mosquitoes are collected using a variety of trapping techniques and are used to identify the mosquito species and primary vector species present in an area and the relative density of those species. When coupled with virus detection protocols, mosquito collections can be screened for the presence of virus and provide a quantifiable index of WNV activity. Adequate sampling requires trapping regularly at representative sites throughout a community, and rapid testing of collections of sufficient size to detect low infection rates in the vector population. Minimally, adult mosquito density (number collected per trap night) and infection rate (number of individual mosquitoes estimated containing WNV per 1,000 specimens tested) should be recorded for each area to provide a basis for tracking mosquito density and virus incidence.
- 2) Larval mosquitoes are collected by taking dip samples from a variety of habitats to identify species present in the area and to identify mosquito sources. Thorough mapping of larval habitats will facilitate larval control or source reduction activities. In addition, where larval management is not feasible, quantitative estimates of larval densities will permit anticipation of new adult emergences. Minimally, the number of larvae

collected per dip and location where collected should be recorded to provide a basis for tracking larval production and association of larval density with resulting adult mosquito population density.

b) Recent experience

- 1) If mosquito trapping effort is intensive, detection of WNV in mosquitoes might precede detection of virus activity by other surveillance tools. If mosquito trapping effort is inadequate, WNV-positive mosquitoes may not be detected prior to the identification of a virus in dead bird, sentinel animal, or human WNV disease cases.
- 2) Moderate to high infection rates sustained for several weeks in *Cx. pipiens* or *Cx. quinquefasciatus* have been associated with subsequent human outbreaks. Sustained high infection rates early in the year are associated with a higher risk for subsequent outbreaks.
- 3) Several intense, focal outbreaks during 2002 were associated with relatively low vector densities, but with high infection rates in key vector species (i.e., infection rates in *Cx. pipiens* or *Cx. quinquefasciatus* of approximately 10 per 1,000 or greater).
- 4) Large numbers of WNV-positive *Cx. tarsalis* pools have been found in association with WNV activity in areas where this species is common. Meaningful infection rates have not yet been determined.
- 5) Avian epizootics may occur without demonstrable human WNV infection. The epizootics are demonstrated, in part, by detection of WNV-positive mosquito pools containing only species that feed predominantly on birds (e.g., *Cx. restuans*).
- 6) During 1999-2002, WNV was detected in 36 mosquito species in the U.S. (see [www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm](http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm)). The vast majority of isolates came from *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. restuans*. Numerous isolates have also come from several potential accessory vectors (i.e., *Cx. tarsalis*, *Cx. salinarius*, *Oc. Ae. albopictus*, *Oc. triseriatus*, *Ae. vexans*, *Cx. nigripalpus*). While detection of WNV in these species demonstrates intensified virus transmission (i.e., virus in primarily mammal-feeding or opportunistic mosquitoes), the contribution of these species to human risk is poorly understood.

c) Advantages of mosquito-based surveillance include the following:

- 1) It may provide the earliest evidence of transmission in an area.
- 2) It helps establish information on potential mosquito vector species.
- 3) It provides an estimate of vector species abundance.
- 4) It gives quantifiable information on virus infection rates in different mosquito species.

- 5) It provides quantifiable information on potential risk to humans and animals.
  - 6) It provides baseline data that can be used to guide emergency control operations.
  - 7) It allows evaluation of control methods.
- d) Disadvantages of mosquito-based surveillance include the following:
- 1) It is labor-intensive and expensive.
  - 2) Substantial expertise is required for collecting, handling, sorting, species identification, processing, and testing.
  - 3) Collectors may be at risk from mosquito bites, especially if day biting species are important bridge vectors, and should wear topical repellents and/or repellent-treated clothing when working in areas where a risk of WNV transmission exists.
- e) Minimal components of an entomological surveillance program

A comprehensive mosquito surveillance program must include larval and adult sampling components, a mapping/record keeping component, a virus-testing component, and a data analysis component. To provide useful data, the surveillance program must be sustained and maintain a consistent effort over several seasons. The exact design of mosquito-based surveillance programs will vary by geography and availability of financial and personnel resources. Not every community will be able to support a comprehensive mosquito-based surveillance program. Minimally, a mosquito-based WNV surveillance program must include the following:

- 1) Collection of adult mosquitoes using gravid traps and/or light traps, providing representative geographic coverage and with sufficient trap sites and trapping frequency to obtain sample sizes required to detect WNV at relatively low infection rates. Use both fixed and flexible trap positions if possible.
  - (a) Fixed positions allow for the development of a database that would let public health officials compare population data to previous years and spatially map changes in mosquito abundance.
  - (b) Flexible sites allow for response to epidemiological and natural events (e.g., a suspected human case, dead crow, or a flood).
  - (c) A variety of trapping methods should be used, including the following:
    - (i) CDC light traps baited with CO<sub>2</sub> for sampling potential accessory vectors.
    - (ii) Gravid traps for *Cx. pipiens* and *Cx. quinquefasciatus* to sample primary WNV vectors.
  - (d) Trap distribution will be influenced by the following species factors:
    - (i) Habitat diversity, size, and abundance;
    - (ii) Resource availability;

- (iii) Proximity to human population centers and/or recreational areas;  
and
  - (iv) Flight range of vector species in the area.
- 2) Laboratory support to identify the mosquitoes' species, and to test the specimens for the presence of WNV. Determine infection rates by species.
    - (a) Make arrangements with a lab for testing. Rapid turnaround is essential.
    - (b) Focus initially on *Culex* mosquitoes to provide first indication of WNV presence.
    - (c) Once virus is detected in *Culex* mosquitoes, pool and test all potential vector species with emphasis on incriminated or suspected species.
  - (3) Data management and analysis capabilities to allow tracking of adult mosquito densities and infection rates over time and space. Patterns of virus activity are more likely to be useful than predetermined threshold levels.
  - (4) Development of a plan with descriptions of actions that will be taken in response to indicators of WNV activity.

## **B. Surveillance for Human Cases**

Because the primary public health objective of surveillance systems for neurotropic arboviruses is prevention of human infections and disease, human case surveillance alone should not be used for the detection of arbovirus activity, except in jurisdictions where arbovirus activity is rare, or resources to support avian-based and/or mosquito-based arbovirus surveillance are unavailable.

GOALS OF SURVEILLANCE FOR HUMAN CASES: To 1) assess the local, state and national public health impact of WNV disease and monitor national trends; 2) demonstrate the need for public health intervention programs; 3) allocate resources; 4) identify risk factors for infection and determine high-risk populations; 5) identify geographic areas in need of targeted interventions; and 6) identify geographic areas in which it may be appropriate to conduct analytic studies of important public health issues.

### **1. Recent Experience**

- a) In the U.S. during 1999-2002, the peak human risk for WN viral infection occurred in August and September, although in 2002 human illness onset was reported as early as mid-May and as late as mid-December. In many regions, the peak minimum infection rates in mosquitoes and a rapid increase in the number of reported avian and equine WN viral infections occurred just prior to the period of maximal human risk.
- b) In 1999-2002, the majority of reported, confirmed, or probable cases of human WN viral disease were among persons with meningoencephalitis. Testing of patients with aseptic meningitis or unexplained febrile illnesses for evidence of

WN viral infection may be beneficial, but can also overwhelm laboratory testing capacity and appears to be of relatively low yield for surveillance purposes since the majority of these cases will not be due to WNV infection.

- c) Most patients with WN encephalitis or meningitis (WNME) are older adults, generally over 50 years old. In the U.S. in 1999-2001, the median age among the 142 reported WNME cases was 68 years. In 2002, among 2,942 reported cases of WN meningoencephalitis, the median age was 59 years. Although 21% of reported cases were in persons younger than 40, only 4% of reported cases were in persons younger than 18.
- d) When WN viral infections were first identified in the U.S., WN encephalitis was associated with a Guillain-Barré-like syndrome with generalized muscle weakness. In 1999-2000, generalized muscle weakness was reported in 29% of WN encephalitis cases. In 2002, at least 2 new neurologic syndromes associated with WN viral infection were identified: acute flaccid paralysis (“WN poliomyelitis-like syndrome”) and brachial plexopathy.
- e) Using CDC-recommended test methods in public health laboratories, WNV-specific IgM antibody was detected in acute-phase (i.e., those collected 8 or less days after illness onset) serum or CSF specimens, or both, in the large majority of confirmed cases. In contrast, only a small minority of suspected cases were subsequently confirmed in which specific IgM antibody reactivity in acute-phase serum or CSF was in the equivocal or low-positive range.
- f) Longitudinal studies of WNME cases have shown that WNV-specific IgM antibody can persist in serum for 12 months or longer.<sup>43</sup> Thus, the presence of WNV-specific IgM antibody in a single serum sample is not necessarily diagnostic of *acute* WN viral infection. For this reason, especially in areas where WNV is known to have circulated previously, suspected, acute WN viral disease cases should be confirmed by observing a fourfold or more change in titer of WNV-specific antibody in serum and the presence of WNV-specific IgM antibody in CSF, when available.
- g) In 1999 in the U.S., the sensitivity of polymerase chain reaction (PCR) tests of CSF for the diagnosis of human WN encephalitis cases was only 57%; more recent statistics are currently unavailable. Thus, PCR for the diagnosis of WN viral infections of the human central nervous system (CNS) continues to be experimental and should not replace tests for the detection of WNV-specific antibody in CSF and serum, tests that are far more sensitive.
- h) During 1999-2001, 7 cases of uncomplicated WN fever (WNF) were reported in the U.S., which represents 5% of the total number of WNV disease cases reported. In 2002, over 1,100 WNF cases were reported (30% of total). Contributing factors likely include the intensive media attention paid to the 2002 epidemic that may have led to increased consumer demand for WNV diagnostic testing by patients and physicians, and the greater availability of commercial testing. Nevertheless, during 1999-2002, WNF was probably significantly

underdiagnosed in the U.S. It has been estimated that approximately 20 WNF cases occur for every WNME case.<sup>44</sup>

- i) For suspected WNV disease cases in immunocompromised patients, WNV-specific antibody may not be present. Since longer viremias may be observed in these patients, testing serum and CSF samples for the presence of virus or viral RNA may be useful.

## 2. Types of Surveillance

### a) Clinical syndromes to monitor

Monitoring of encephalitis cases is the highest priority. Monitoring milder illnesses (e.g., aseptic meningitis, Guillain-Barré syndrome, acute flaccid paralysis, and brachial plexopathy, and fever or rash illnesses) is resource-dependent and should be of lower priority.

### b) Types of human surveillance

#### 1) *Enhanced passive surveillance*

In the absence of known WNV activity in an area, enhanced passive surveillance\* for hospitalized cases of encephalitis (and milder clinical syndromes as resources allow\*\*), and for patients who have IgM antibodies to either WN or SLE virus in tests conducted in diagnostic or reference laboratories, should be employed. A high clinical suspicion for arboviral encephalitis should be encouraged among health care providers. When the diagnosis is in doubt, appropriate clinical specimens should be submitted to CDC or another laboratory capable of performing reliable serologic testing for antibodies to domestic arboviruses. Testing of CSF and paired acute- and convalescent-phase serum samples should be strongly encouraged to maximize the accuracy of serologic results.

#### 2) *Active surveillance*

Active surveillance should be strongly considered in areas with known WNV activity. In general, one or both of the following approaches should be taken: (a) Contact physicians in appropriate specialties (i.e., infectious diseases, neurology, and critical care) and hospital infection control personnel on a regular basis to inquire about patients with potential arboviral infections; (b) Implement laboratory-based surveillance to identify CSF specimens

---

\* Passive surveillance enhanced by general alerts to key health care personnel such as primary care providers, infectious disease physicians, neurologists, hospital infection control personnel, and diagnostic laboratories.

\*\* While human infections with neurotropic arboviruses are usually clinically inapparent, most clinically apparent infections are associated with fever, with or without neurologic manifestations, which can range from mild aseptic meningitis to fulminant and fatal encephalitis. Signs and symptoms may include fever, headache, stiff neck, confusion or other mental status changes, nausea, vomiting, meningismus, cranial nerve abnormalities, paresis or paralysis, sensory deficits, altered reflexes, abnormal movements, convulsions, and coma of varying severity. Arboviral meningitis or encephalitis cannot reliably be clinically distinguished from other central nervous system infections.

meeting sensitive but nonspecific criteria for arboviral infections (e.g., mild to moderate pleocytosis and negative tests for the presence of nonarboviral agents such as bacteria, fungi, herpesviruses, and enteroviruses) and test them for evidence of WNV infection.

### 3) *Special surveillance projects*

Special projects may be used to enhance arboviral disease surveillance. Such projects include the Emerging Infections Network of the Infectious Diseases Society of America (IDSA EIN), Emergency Department Sentinel Network for Emerging Infections (EMERGENCY ID NET), Unexplained Deaths and Critical Illnesses Surveillance of the Emerging Infections Programs (EIP), and the Global Emerging Infections Sentinel Network of the International Society of Travel Medicine (GeoSentinel). In some areas, syndromic surveillance systems may be considered. “Piggy-backing” surveillance for WNME and milder clinical forms of WN viral infection, such as fever with rash or lymphadenopathy, onto existing syndromic surveillance systems, especially those involving large health maintenance organizations, may be considered. Real-time computerized syndromic surveillance in emergency departments, and special surveillance projects to identify WNV disease in pediatric populations, may be useful.

## 3. Specimens

### a) Cerebrospinal fluid (CSF)

In WNME cases, WNV-specific IgM antibody commonly can be found in CSF on the day of illness onset using antibody-capture ELISA. Virus also may be isolated (rarely) or detected by RT-PCR (in up to 60% of cases) in acute-phase CSF samples.

### b) Serum

Paired acute-phase (collected 0-8 days after onset of illness) and convalescent-phase (collected 14-21 days after the acute specimen) serum specimens are useful for demonstration of seroconversion to WNV and other arboviruses by ELISA or neutralization tests. Although tests of a single acute-phase serum specimen may provide evidence of a recent WNV infection, a negative acute-phase specimen is inadequate for ruling out such an infection, underscoring the importance of collecting paired samples. As mentioned previously, antibody synthesis in immunocompromised individuals might be delayed or absent altogether.

### c) Tissues

When arboviral encephalitis is suspected in a patient who undergoes a brain biopsy or who dies, tissues (especially brain samples, including samples of cortex, midbrain, and brainstem), heart/venous blood, and buffy coat samples should be submitted to CDC or other specialized laboratories for arbovirus and other testing. Tissue specimens should be divided; half should be frozen at -70°C and the other half fixed in formalin. Available studies include gross pathology, histopathology, RT-PCR tests, virus isolation, and immunohistochemistry.

#### **4. Surveillance Case Definition**

The national case definition for arboviral encephalitis (available at [www.cdc.gov/epo/dphsi/casedef/encephalitiscurrent.htm](http://www.cdc.gov/epo/dphsi/casedef/encephalitiscurrent.htm)) should be used to classify cases as confirmed or probable, once appropriate laboratory results are available (also see Section II). In CDC publications of national arbovirus surveillance data, no distinction is usually made between confirmed and probable human cases for the purposes of case counting.

#### **5. Minimal Components of a Human Surveillance System**

Enhanced passive surveillance for hospitalized encephalitis cases of unknown etiology, and for patients who have IgM antibodies to either WN or SLE virus in tests conducted in diagnostic or reference laboratories.

### ***C. Geography and Timing***

In general, the WNV transmission season in the U.S. is longer than that for other domestic arboviruses and requires longer periods of ecologic and human surveillance.

#### **1. Northeastern and Midwestern U.S.**

In the northeastern states in 2001-2002, human illness onset occurred as early as early July and as late as mid-November. During these same years, avian cases occurred as early as the first week of April and as late as the second week of December. Active ecological surveillance and enhanced passive surveillance for human cases should begin in early spring and continue through the fall until mosquito activity ceases because of cold weather. Surveillance in urban and suburban areas should be emphasized.

#### **2. Southern U.S.**

In 2001-2002, WNV circulated throughout the year, especially in the Gulf states. Although, in 2001-2002, human illness onset was reported as early as mid-May and June and as late as mid-December, equine and avian infections were reported in all months of the year. Active ecologic surveillance and enhanced passive surveillance for human cases should be conducted year round in these areas.

#### **3. Western U.S.**

In 2002, WNV activity was first reported among humans and animals in Rocky Mountain states and among animals in Pacific coast states. These events occurred relatively late in the year (mid-August). Predicting the temporal characteristics of future WNV transmission seasons based on these limited reports is not possible. Despite this limitation, active ecological surveillance and enhanced passive surveillance for human cases beginning in early spring and continuing through the fall until mosquito activity ceases because of cold weather should be encouraged.

#### **4. Other Areas of the Western Hemisphere**



In 2002, Canada experienced a WNV epidemic in Ontario and Quebec provinces and an equine/avian epizootic that extended from the maritime provinces to Saskatchewan.

Recent serologic evidence supports the conclusion that WNV has now reached Central America. Further spread to South America by migratory birds seems inevitable, if this has not already occurred. Development of surveillance systems capable of detecting WNV activity should be encouraged in the Caribbean and Central and South America. WNV surveillance should be integrated with dengue surveillance in these areas, and with yellow fever surveillance in areas where urban or peri-urban transmission of this virus occurs.

## II. **LABORATORY DIAGNOSIS**

The clinical presentation of most patients with viral encephalitis is similar regardless of the cause. Also, infection by many of the arboviruses that cause encephalitis, including West Nile and St. Louis encephalitis viruses, usually is clinically inapparent, or causes a nonspecific viral syndrome in most patients. Definitive diagnosis, therefore, can only be made by laboratory testing using specific reagents. To be successful, active surveillance must have adequate laboratory support.

The basic laboratory diagnostic tests—and how they should be used at the national, state, and local level—are outlined below. The initial designation of reference and regional laboratories that can do all testing will be based on the availability of biosafety level 3 (BSL3) containment facilities. Details of the surveillance case definition for human West Nile virus (WNV) disease and of how the laboratory diagnostic tests are used to support surveillance are presented in Appendix B.

### **A. Biocontainment**

#### **1. Laboratory Safety Issues**

Laboratory-associated infections with WNV have been reported in the literature. The Subcommittee on Arbovirus Laboratory Safety (SALS) in 1980, reported 15 human infections from laboratory accidents. One of these infections was attributed to aerosol exposure. Recently, two parenteral inoculations have been reported during work with animals.

- a) WNV may be present in blood, serum, tissues and CSF of infected humans, birds, mammals and reptiles. The virus has been found in the oral fluids and feces of birds. Parenteral inoculation with contaminated materials poses the greatest hazard; contact exposure of broken skin is a possible risk. Sharps precautions should be strictly adhered to when handling potentially infectious materials. Workers performing necropsies on infected animals may be at high risk of infection.
- b) Biosafety Level 2 practices and facilities are recommended for activities for human diagnostic specimens. In some cases it may be advisable to perform initial processing of clinical samples in a biosafety cabinet, particularly if high levels of virus is suspected (such as tissues from fatal human cases). Biosafety Level 2 is recommended for processing field collected mosquito pools. Biosafety Level 3 and Animal Biosafety Level 3 practices, containment equipment, and facilities are recommended, respectively, for all manipulations of West Nile cultures and for experimental animal and vector studies. Containment specifications are available in the Centers for Disease Control and Prevention/National Institutes of Health publication Biosafety in Microbiological and Biomedical Laboratories (BMBL).<sup>45</sup> This document can be found online at both <http://bmbi.od.nih.gov/> and <http://www.cdc.gov/od/ohs/biosfty/bmbi4/bmbi4toc.htm>.
- c) All bird necropsies should be done in a Class 2 biological safety cabinet.

## 2. Shipping of Agents

Shipping and transport of WNV and clinical specimens should follow current International Air Transport Association (IATA) and Department of Commerce recommendations. Because of the threat to the domestic animal population, a U.S. Department of Agriculture (USDA) shipping permit is required for transport of known WNV isolates. For more information, visit the IATA dangerous goods Web site at <http://www.iata.org/cargo/dg/>, and the USDA Animal and Plant Health Inspection Service (APHIS), National Center for Import /Export's Web site at <http://www.aphis.usda.gov/ncie/>.

### B. Serologic Laboratory Diagnosis

Accurate interpretation of serologic findings requires knowledge of the specimen. For human specimens the following data must accompany specimens submitted for serology before testing can proceed or results can be properly interpreted and reported: 1) symptom onset date (when known); 2) date of sample collection; 3) unusual immunological status of patient (e.g., immunosuppression); 4) state and county of residence; 5) travel history in flavivirus-endemic areas; 6) history of prior vaccination against flavivirus disease (e.g., yellow fever, Japanese encephalitis, or Central European encephalitis); and 7) brief clinical summary including clinical diagnosis (e.g., encephalitis, aseptic meningitis).

#### 1. Human

- a) Commercial kits for human serologic diagnosis of WNV infection are currently in development. Until these kits are available, the CDC-defined IgM and IgG ELISA should be the front-line tests for serum and CSF.<sup>46-48</sup> These ELISA tests are the most sensitive screening assays available. The HI and indirect immunofluorescent antibody (IFA) test may also be used to screen samples for flavivirus antibodies. Laboratories performing HI assays need be aware that the recombinant WNV antigens produced to date are not useful in the HI test; mouse brain source antigen (available from CDC) must be used in HI tests. The recombinant WNV antigen is available from commercial sources.
- b) To date, the prototype WNV strains Eg101 or NY99 strains have performed equally well as antigens in diagnostic tests for WNV in North America.
- c) To maintain Clinical Laboratory Improvements Amendments (CLIA) certification, CLIA recommendations for positive and negative ranges should be followed, and laboratories doing WNV testing should participate in a proficiency testing program through experienced reference laboratories; CDC's Division of Vector-Borne Infectious Diseases in Fort Collins, Colorado and the National Veterinary Services Laboratories in Ames, Iowa both offer this type of program.
- d) Because the ELISA can cross-react between flaviviruses (e.g., SLE, dengue, yellow fever, WN), it should be viewed as a screening test only. Initial serologically positive samples should be confirmed by neutralization test. Specimens submitted for arboviral serology should also be tested against other arboviruses known to be active or be present in the given area (e.g., test against SLE, WN and EEE viruses in Florida).

## **2. Animal**

- a) In general, the procedures for animal serology should follow those used with humans cited above.
- b) Plaque-reduction neutralization test (PRNT) and HI assays, although technically more demanding, may be useful because they are species independent.

### **C. Virologic Laboratory Diagnosis**

Experience gained in WNV diagnostic testing over the past 4 years has led to the following recommendations:

#### **1. Virus Isolation**

- a) Virus isolation attempts should be performed in known susceptible mammalian or mosquito cell lines. Mosquito origin cells may not show cytopathic effect and should be screened by immunofluorescence.
- b) Appropriate samples for virus isolation are prioritized as follows:
  - 1) Clinically ill humans - CSF (serum samples may be useful early in infection)
  - 2) Human (biopsy or postmortem) - brain tissue
  - 3) Horses (postmortem) - brain tissue (including brainstem), spinal cord tissue
  - 4) Birds - kidney, brain, heart
  - 5) Other mammals - multiple tissues, especially kidney and brain
- c) Confirmation of virus isolate identity can be accomplished by indirect immunofluorescence assay (IFA) using virus-specific monoclonal antibodies, nucleic acid detection, or virus neutralization.
- d) The IFA using well-defined murine monoclonal antibodies (MAbs) is the most efficient, economical, and rapid method to identify flaviviruses. MAbs are available that can differentiate WNV and SLE virus from each other and from other flaviviruses. Flavivirus-grouping MAbs are available for use as positive controls, and MAbs specific for other arboviruses can be used as negative controls. In addition, incorporating MAbs specific for other arboviruses known to circulate in various regions will increase the rapid diagnostic capacities of state and local laboratories. These reagents are available and should be used.
- e) Nucleic acid detection methods including RT-PCR, TaqMan and nucleic acid sequence based amplification (NASBA) methods may be used to confirm virus isolates as WNV.
- f) Virus neutralization assays also may be used to differentiate viruses, by using fourfold or greater titer differences as the diagnostic criterion in paired specimens (acute- and convalescent-phase).

## 2. Virus Detection in Tissues

### a) Antigenic analysis

- 1) Immunohistochemistry (IHC) using virus-specific MAbs on brain tissue has been very useful in identifying both human and avian cases of WNV infection. In suspected fatal cases, IHC should be performed on formalin-fixed autopsy, biopsy, and necropsy material, ideally collected from multiple anatomic regions of the brain, including the brainstem, midbrain, and cortex.<sup>24, 49</sup>
- 2) Well-characterized antigen-capture ELISAs are now available for detection of SLE<sup>50,51</sup> and WNV antigen in mosquito pools and avian tissues.<sup>25</sup>

### b) Nucleic acid analysis

A number of nucleic acid detection methods have recently been employed for WNV diagnostic and surveillance purposes. An independent antigen or nucleic acid test is required to confirm detection of WNV nucleic acid with any of these methods.

- 1) RT-PCR of tissues, mosquito pools, and CSF has proven to be a useful surveillance tool. RT-nested PCR has detected WNV nucleic acid in equine brain and spinal cord tissues. Standardized protocols developed by reference laboratories should be disseminated, and primer design information should be included so that other laboratories can prepare primers. A proficiency testing program should be developed by the reference laboratories so that these tests can be CLIA-certified in local laboratories.
- 2) Fluorogenic 5' nuclease techniques (real-time PCR) and nucleic acid sequence-based amplification (NASBA) methods have been developed and have undergone initial validation in specific diagnostic applications.<sup>24,52-54</sup>

## D. *Training and Infrastructure*

### 1. **State and Local Arbovirus Laboratories**

Greater numbers of capable state and local laboratories performing screening assays (such as ELISA) should be developed to reduce time demands on reference laboratories. Reference laboratories should be utilized to confirm results of state and local laboratories, particularly for the initial identification of WNV in new locations and in new hosts.

### 2. **Training Programs**

Laboratory training programs have been developed and implemented at the federal level. Additional regional training programs may be beneficial.

### **III. PREVENTION AND CONTROL**

Prevention and control of arboviral diseases is accomplished most effectively through a comprehensive, integrated mosquito management program using sound integrated pest management (IPM) principles.<sup>55</sup> IPM is based on an understanding of the underlying biology of the transmission system, and utilizes regular monitoring to determine if and when interventions are needed to keep pest numbers below levels at which intolerable levels of damage, annoyance, or disease occur. IPM-based systems employ a variety of physical, mechanical, cultural, biological and educational measures, singly or in appropriate combination, to attain the desired pest population control.

Programs consistent with best practices and community needs should be established at the local level and, at a minimum, should be capable of performing surveillance sensitive enough to detect West Nile Virus (WNV) enzootic/epizootic transmission that has been associated with increased risk of disease in humans or domestic animals. Integrated mosquito management programs designed to minimize risk of WNV transmission and prevent infections of humans and domestic animals should optimally include the following components (modified from information provided by the American Mosquito Control Association, the New Jersey Mosquito Control Association, and the Florida Coordinating Council on Mosquito Control)<sup>56-58</sup>

#### **A. Surveillance**

Effective mosquito control begins with a sustained, consistent surveillance program that targets pest and vector species, identifies and maps their immature habitats by season, and documents the need for control. Records should be kept on the species composition of mosquito populations prior to enacting control of any kind and to allow programs to determine the effectiveness of control operations. All components of the integrated management program must be monitored for efficacy using best practices and standard indices of effectiveness. The following is a list of surveillance methodologies used by mosquito control agencies.

##### **1. Larval Mosquito Surveillance**

Larval surveillance involves sampling a wide range of aquatic habitats for the presence of pest and vector species during their developmental stages. Most established programs have a team of trained inspectors to collect larval specimens on a regular basis from known larval habitats, and to perform systematic surveillance for new sources. A mosquito identification specialist normally identifies the larvae's species. Properly trained mosquito identification specialists can separate nuisance and vector mosquito species. Responsible control programs target vector and nuisance populations for control and avoid managing habitats that support benign species.

##### **2. Adult Mosquito Surveillance**

Adult mosquito surveillance is used to monitor species presence and relative abundance of adult mosquitoes in an area. Information derived from adult mosquito surveillance programs using standardized and consistent surveillance efforts provide information essential to monitoring potential vector activity, setting action thresholds, and evaluating control efforts. Various methods are available for this purpose and

have been demonstrated to be effective in collecting a variety of mosquito species.<sup>59</sup> The New Jersey light trap, CDC's miniature light trap, and other modifications of this design, with or without carbon dioxide bait, have been used extensively for collecting host-seeking adult mosquitoes.<sup>60</sup> Gravid traps frequently are used to monitor the ovipositing segment of *Cx. pipiens* and *Cx. restuans* populations. These species have been incriminated as the primary enzootic vectors of WNV in the northeastern states.<sup>61,62</sup> Host-seeking *Cx. tarsalis*, a species that has been strongly associated with WNV transmission in areas where this species is common, are readily collected in CO<sub>2</sub>-baited CDC miniature light traps. Resting boxes frequently are used to measure populations of *Culiseta melanura*, a bird-feeding mosquito that is important in the amplification of eastern equine encephalitis (EEE) virus. Pigeon-baited traps may be employed to measure host-seeking *Culex* mosquitoes that amplify St. Louis encephalitis (SLE) and West Nile viruses. Day-active mosquitoes like *Ae. albopictus* are difficult to collect, and obtaining a sample representative of the local populations requires extra effort. Where these species are important, sample sizes may be enhanced by using CO<sub>2</sub>-baited CDC miniature light traps during daylight hours or by using alternative trap configurations that may be more effective in collecting these species (e.g., Fay trap or traps using a counterflow geometry). Trap deployment should carefully address species habitat requirements on several spatial scales.

### 3. Virus Surveillance

The purpose of this component of the vector management program is to determine the prevalence of WNV in the mosquito population. This is often expressed simply as the number of WNV-positive mosquito pools of a given species collected at a defined location and time period. While the number of positive pools provides valuable information, it does not provide an index of virus prevalence in the vector population. Preferably, the proportion of the mosquito population carrying the virus should be expressed as the infection rate (IR, expressed as the estimated number of infected individual mosquitoes per 1,000 specimens tested). This is a more useful index of virus prevalence. The IR can be calculated by dividing the number of positive pools by the total number of specimens tested for that species and collection period, and multiplying the proportion by 1,000. This assumes that a positive pool contains only one infected mosquito, which is a valid assumption in most circumstances. When infection rates are high or sample sizes are low, a more accurate estimate of IR may be obtained by using a maximum likelihood estimate of the infection rate – see [www.cdc.gov/ncidod/dvbid/westnile/software.htm](http://www.cdc.gov/ncidod/dvbid/westnile/software.htm). Elevated infection rates, particularly if sustained over several weeks or in populations of opportunistic blood-feeders that may act as bridge vectors, are indicators of increased WNV transmission risk. Specimens collected in the routine adult mosquito surveillance program plus special collections from key areas identified by other surveillance indicators (e.g., dead birds, sentinel flocks) can be used for this purpose. Mosquito collections made at permanent study sites in a sustained program provide important baseline data to which new surveillance data are compared and decisions about human risk and need for emergency interventions are made.

### B. Source Reduction

Source reduction is the alteration or elimination of mosquito larval habitat breeding. This remains the most effective and economical method of providing long-term mosquito

control in many habitats. Source reduction can include activities as simple as the proper disposal of used tires and the cleaning of rain gutters, bird baths and unused swimming pools by individual property owners, to extensive regional water management projects conducted by mosquito control agencies on state and/or federal lands. All of these activities eliminate or substantially reduce mosquito breeding habitats and the need for repeated applications of insecticides in the affected habitat. Source reduction activities can be separated into the following two general categories:

## **1. Sanitation**

The by-products of human's activities have been a major contributor to the creation of mosquito breeding habitats. An item as small as a bottle cap or as large as the foundation of a demolished building can serve as a mosquito breeding area. Sanitation, such as tire removal, stream restoration, catch-basin cleaning and container removal, is a major part of all integrated vector management programs. Mosquito control agencies in many jurisdictions have statutory powers that allow for due process and summary abatement of mosquito-related public health nuisances created on both public and private property. The sanitation problems most often resolved by agency inspectors are problems of neglect, oversight, or lack of information on the part of property owners. Educational information about the importance of sanitation in the form of videos, slide shows, and fact sheets distributed at press briefings, fairs, schools and other public areas are effective.

## **2. Water Management**

Water management for mosquito control is a form of source reduction that is conducted in fresh and saltwater breeding habitats. Water management programs for vector control generally take two forms, described below. Water management through impoundment and open water management have been very effective in the past. Recently, restrictions on modification of aquatic habitats have limited the implementation of these practices, and in many areas, water management for vector control is no longer routinely employed and may be impractical in many settings. In these situations, alternative methods of mosquito management must be employed.

### **a) Impoundment Management**

Impoundments are mosquito-producing marshes around which dikes are constructed, thereby allowing water to stand or to be pumped onto the marsh surface from the adjacent estuary. This eliminates mosquito oviposition sites on the impounded marsh and effectively reduces their populations. Rotational Impoundment Management (RIM) is the technique developed to minimally flood the marsh during the summer months and then use flapgated culverts to reintegrate impoundments to the estuary for the remainder of the year, thereby allowing the marsh to provide many of its natural functions. Although impoundments usually achieve adequate control of salt-marsh mosquitoes, there are situations in which impoundments can collect stormwater or rainwater and create freshwater mosquito problems that must be addressed using other techniques.

### **b) Open Marsh Water Management (OMWM)**



Ditching as a source-reduction mosquito control technique has been used for many years. Open marsh water management is a technique whereby mosquito-producing locations on the marsh surface are connected to deep-water habitat (e.g., tidal creeks, deep ditches) with shallow ditches. Mosquito broods are controlled without pesticide use by allowing larvivorous fish access to mosquito-producing depressions. Conversely, the draining of these locations occurs before adult mosquitoes can emerge. OMWM can also include establishing or improving a hydrological connection between the marsh and estuary, providing natural resource enhancement as well as mosquito control benefits. The use of shallow ditching (ditches approximately 3 feet or less in depth rather than the deep ditching used in years past) is considered more environmentally acceptable because than deep ditching because fewer unnatural hydrological impacts occur to the marsh.

c) Management in Stormwater Retention Structures

Source reduction and water management practices may also be applied to stormwater retention structures designed to hold runoff before it is discharged into groundwater or surface water. Mosquito control should be considered in the design, construction, and maintenance of these structures, as appropriate. Stormwater retention structures should be designed in consultation with experts in mosquito biology and control to prevent as much mosquito production as possible, and to facilitate proper functioning and maintenance in the future. Regulations associated with stormwater retention and flood control structures should incorporate appropriate operations and maintenance provisions including considerations for routine monitoring and control of mosquito populations.

## **C. Chemical Control**

Insecticides can be directed against either the immature or adult stage of the mosquito life cycle when source reduction and water management are not feasible or have failed because of unavoidable or unanticipated problems, or when surveillance indicates the presence of infected adult mosquitoes that pose a health risk.<sup>63</sup> Chemicals used by mosquito control agencies must comply with state and federal requirements. Public health pesticide applicators and operators in most states are required to be licensed or certified by the appropriate state agencies.

### **1. Larviciding**

Larviciding, the application of chemicals to kill mosquito larvae or pupae by ground or aerial treatments, is typically more effective and target-specific than adulticiding, but less permanent than source reduction. An effective larviciding program is an important part of an integrated mosquito control operation. The objective of larviciding is to control the immature stages at the breeding habitat before adult populations have had a chance to disperse and to maintain populations at levels at which the risk of arbovirus transmission is minimal. Larvicides can be applied from the ground or by aerial application if large or inaccessible areas must be treated. Several materials in various formulations are labeled for mosquito larviciding including the organophosphate temephos (Abate); several biological larvicides such as *Bacillus thuringiensis israelensis* (*Bti*, a bacterial larvicide), *Bacillus sphaericus*; methoprene, an insect growth regulator (e.g., Altosid,); several larvicidal oils (e.g.,

petroleum-based Golden Bear and mineral-based Bonide) and monomolecular surface films (e.g., Agnique, Arosurf); and in some limited habitats diflubenzuron (e.g., Dimilin, a chitin synthesis inhibitor). Applications of larvicides often encompass fewer acres than adulticides because treatments are made to relatively small areas where larvae are concentrated, as opposed to larger regions where adults have dispersed. When applying larvicides, it is important that the material be specific for mosquitoes, minimize impacts on non-target organisms, and, where appropriate, be capable of penetrating dense vegetation canopies. Larvicide formulations (i.e., liquid, granular, solid) must be appropriate to the habitat being treated, accurately applied, and based on surveillance data. Accuracy of application is important because missing even a relatively small area can cause the emergence of a large mosquito brood resulting in the need for broad-scale adulticiding.

## 2. Adulticiding

Adulticiding is the application of pesticides to kill adult mosquitoes. The ability to control adult mosquitoes is an important component of any integrated mosquito management program, and like the other components of the program, its use should be based on surveillance data. Mosquito adulticiding may be the only practical control technique available in situations where surveillance data indicate that is necessary to reduce the density of adult mosquito populations quickly to lower the risk of WNV transmission to humans. In some situations, source reduction and larvicide applications are not practical, and adulticide application is the only available control strategy. Mosquito adulticides typically are applied as an Ultra-Low-Volume (ULV) spray where small amounts of insecticide are dispersed either by truck-mounted equipment or from fixed-wing or rotary aircraft.<sup>64-68</sup> Thermal fog applications of adulticides by ground or air are also used in some areas, but to a much lesser degree. Barrier treatments, typically applied as high volume liquids with hand-held spray equipment using compounds with residual characteristics, are common in some U.S. locations. This technique is especially attractive to individual homeowners living near mosquito-producing habitats where residual chemicals applied along property boundaries can provide some control benefits. Mosquito adulticiding differs fundamentally from techniques used to control many other adult insects. For adult mosquito control, insecticide must drift through the habitat in which mosquitoes are flying in order to provide optimal control benefits. The EPA has determined that the insecticides labeled nationally for this type of application do not pose unreasonable health risks to humans, wildlife, or the environment when used according to the label.<sup>56</sup> Adulticides labeled for mosquito control include several organophosphates such as malathion and naled. Some natural pyrethrins and synthetic pyrethroids (permethrin, resmethrin and sumithrin) also hold adulticide labels. Insecticide selection and timing of application should be based on the distribution and behavior of the target mosquito species. Application of adulticides should be timed to coincide with the activity period of the target mosquito species. Many *Culex* species are nocturnal and are active in the tree canopy level. This should be taken into consideration when planning adulticide applications. Operational experience indicates that *Cx. pipiens* and *Cx. quinquefasciatus* may require more frequent adulticide application to achieve desired levels of population reduction during an outbreak. Control of adult day-active species poses additional problems because ULV adulticide effectiveness is greatly reduced during daylight

hours. Early-morning use of adulticides, applied before temperatures rise, may provide a measure of control for these species.

#### **D. Resistance Management**

In order to delay or prevent the development of insecticide resistance in vector populations, integrated vector management programs should include a resistance management component (modified from Florida Coordinating Council on Mosquito Control, 1998).<sup>57</sup> Ideally, this should include annual monitoring of the status of resistance in the target populations to:

1. Provide baseline data for program planning and pesticide selection before the start of control operations.
2. Detect resistance at an early stage so that timely management can be implemented (even detection of resistance at a late stage can be important in elucidating why disease control may fail); however, in such cases, management options other than replacement of the pesticide may not be possible).
3. Continuously monitor the effect of control strategies on resistance. In addition to monitoring resistance in the vector population, the integrated program should include options for managing resistance that are appropriate for the local conditions.<sup>69-70</sup> The techniques regularly used include the following:
  - a) Management by Moderation - preventing onset of resistance by
    - 1) Using dosages no lower than the lowest label rate to avoid genetic selection.
    - 2) Using less frequent applications.
    - 3) Using chemicals of short environmental persistence.
    - 4) Avoiding slow-release formulations.
    - 5) Avoiding the use of the same class of insecticide to control both adults and immature stages.
    - 6) Applying locally. Currently, most districts treat only hot spots. Area-wide treatments are used only during public health alerts or outbreaks.
    - 7) Leaving certain generations, population segments, or areas untreated.
    - 8) Establishing high pest mosquito densities or action thresholds prior to insecticide application.
    - 9) Alternation of biorational larvicides and insect growth regulators annually or at longer intervals.
  - b) Management by continued suppression - a strategy used in areas of high-value (e.g., heavily touristed areas) or where arthropod vectors of disease must be kept at very low densities.

This does not mean saturation of the environment by pesticides, but rather the saturation of the defense mechanisms of the insect by insecticide dosages that can overcome resistance. This is achieved by the application of dosages within label rates but sufficiently high to be lethal to susceptible as well as to

heterozygous-resistant individuals. If the heterozygous individuals are killed, resistance (which is a homozygous trait) will be slow to emerge. This method should not be used if any significant portion of the population in question is resistant. Another approach more commonly used is the addition of synergists that inhibit existing detoxification enzymes and thus eliminate the competitive advantage of these individuals. Commonly, the synergist of choice in mosquito control is piperonyl butoxide (PBO).

- c) Management by multiple attack - achieving control through the action of several different and independent pressures such that selection for any one of them would be below that required for the development of resistance.

This strategy involves the use of insecticides with different modes of action in mixtures or in rotations. There are economic problems (e.g., costs of switching chemicals or having storage space for them) associated with this approach, and critical variables in addition to mode of action must be taken into consideration (i.e., mode of resistance inheritance, frequency of mutations, population dynamics of the target species, availability of refuges, and migration). General recommendations are to evaluate resistance patterns at least annually and the need for rotating insecticides at annual or longer intervals.

#### **E. Biological Control**

Biological control is the use of biological organisms, or their by-products, to control pests. Biocontrol is popular in theory, because of its potential to be host-specific and virtually without non-target effects. Overall, larvivorous fish are the most extensively used biocontrol agent for mosquitoes. Predaceous fish, typically *Gambusia* or other species which occur naturally in many aquatic habitats, can be placed in permanent or semipermanent water bodies where mosquito larvae occur, providing some measure of control. Other biocontrol agents that have been tested for mosquito control, but that to date generally are not widely used, include the predaceous mosquito *Toxorhynchites*, predacious copepods, the parasitic nematode *Romanomermis*, and the fungus *Lagenidium giganteum*. Biocontrol certainly holds the possibility of becoming a more important tool and playing a larger role in mosquito control in the future, but will likely be effective only as part of an integrated approach.

#### **F. Continuing Education of Mosquito Control Workers**

Continuing education is directed toward operational workers to instill or refresh knowledge related to practical mosquito control. Training is primarily in safety, applied technology, and requirements for the regulated certification program mandated by most states.

#### **G. Vector Management in Public Health Emergencies**

A surveillance program adequate to monitor WNV activity levels associated with human risk must be in place. Detection of epizootic transmission of enzootic arboviruses typically precedes detection of human cases by several days to 2 weeks or longer (e.g., as found in SLE epidemics).<sup>71,72</sup> If adequate surveillance is in place, the lead time between detecting significant levels of epizootic transmission and occurrence of human cases can be increased, which will allow for more effective intervention practices.<sup>19,27,31</sup> Early-season detection of enzootic or epizootic WNV activity appears to be correlated

with increased risk of human cases later in the season. Control activity should be intensified in response to evidence of virus transmission, as deemed necessary by the local health departments. Such programs should consist of public education emphasizing personal protection and residential source reduction; municipal larval control to prevent repopulation of the area with competent vectors; adult mosquito control to decrease the density of infected, adult mosquitoes in the area; and continued surveillance to monitor virus activity and efficacy of control measures.

As evidence of sustained or intensified virus transmission in an area increases, emergency response should be implemented. This is particularly important in areas where vector surveillance indicates that infection rates in *Culex* mosquitoes are increasing, or that potential accessory vectors (e.g., mammalophilic species) are infected with WNV. Delaying adulticide applications in such areas until human cases occur is illogical and negates the value and purpose of the surveillance system.

#### **H. Adult Mosquito Control Recommendations**

Ground-based (truck-mounted) application of adult mosquito control agents has several positive attributes. Where road access is adequate, such as in urban and suburban residential areas, good coverage may be achieved. In addition, ground-based application can be done throughout the night, thereby targeting night-active mosquito species. Such applications are prone to skips and patchy coverage in areas where road coverage is not adequate or in which the habitat contains significant barriers to spray dispersal and penetration.

Aerial application is capable of covering larger areas in shorter time periods than a ground-based application. This is a critical positive attribute when large residential areas must be treated quickly. In addition, aerial application is less prone to patchy coverage than ground-based application in areas where road coverage is not adequate.

One limitation of aerial application is that many applicators will not fly at night, potentially reducing the effectiveness of the applications in *Culex* species control efforts.

Cost benefits of aerial application over ground application may not be realized unless relatively large areas are treated.

Several formulations of a variety of active ingredients are available for adulticide applications. Material choice for ground-based or aerially applied mosquito control in public health emergency situations is limited by EPA restrictions on the pesticide label and applicable state and local regulations.

Multiple applications will likely be required to appreciably reduce *Culex* populations and interrupt arbovirus transmission. An emergency SLE virus response plan developed for New Orleans, Louisiana<sup>63</sup> indicates the need for repeated applications to control *Cx. quinquefasciatus*, and the need to repeatedly apply adulticides in high-risk areas (areas with human cases or positive surveillance events). Two to three adulticide applications spaced 3-4 days apart may be required to significantly reduce *Cx. pipiens* populations. Effective surveillance must be maintained to determine if and when re-treatment is required to maintain suppression of the vector populations.

Urban/suburban population centers with multiple positive surveillance events as described above should be treated first to most efficiently protect the largest number of

people from exposure to WNV. Applications should be timed to coincide with the peak activity periods of the target species. For example, applications should be made at night to maximize control of night-active *Culex* species. Other species such as *Oc. sollicitans* or *Ae. vexans* are active shortly after sunset and are effectively controlled with appropriately timed applications. Day-active potential accessory vectors (e.g., *Oc. japonicus*, *Oc. triseriatus*, *Ae. albopictus*) must be addressed separately and are most effectively controlled by residential source reduction efforts, though there is preliminary evidence that early morning ULV applications may be used to control these species.

### **I. Determining the Scope of Mosquito Adulticiding Operations**

Once arbovirus activity is detected in a jurisdiction and a decision is made to implement or intensify mosquito control by using adulticides, the size of the area to be treated must be determined. In the broadest context, the underlying program objective (i.e., interruption of the enzootic transmission cycle vs. prevention of transmission to humans and domestic animals) should determine the amount of adulticide coverage that is required. For most jurisdictions the objective is the prevention of transmission to humans and domestic animals. There is no simple formula for determining how large an area to treat around a positive surveillance indicator or a suspected or confirmed human case of WNV. Nor is there adequate information to guide decisions about the degree of vector population suppression that must be attained, or for how long this suppression must be maintained to reduce human disease risk. At a minimum, the following factors must be considered when deciding the scope of the adulticiding effort:

1. The general ecology of the area, e.g., key habitat types and the presence of natural barriers such as large rivers;
2. The population density, distribution, flight range, and age structure (proportion of parous females) of the target mosquito species;
3. The flight range of the avian amplifying host(s);
4. The length of time since birds started dying or became infected in the affected area (typically, there may be a lag of several weeks between recovery of dead birds and confirmation of WNV infection) or since virus-positive mosquito pools were collected;
5. The human population characteristics – spatial distribution and density relative to the positive locality (e.g., urban vs. rural), age demographics;
6. Evidence of persistent WNV activity detected by the surveillance program; and
7. Season of the year and how long WNV activity can be expected to persist until the epizootic/epidemic vector(s) enter diapause.

Several of these factors will be unknown or poorly understood. Technical assistance from a mosquito control professional, particularly one experienced in mosquito control in the region, is crucial in this process. Practical experience in conducting mosquito control is required to refine control recommendations. For example, the size of an area selected for control applications may be reduced in response to structures like open areas, bodies of water, major highways, or other barriers that may restrict the distribution of targeted species. Alternatively, adulticide coverage may be expanded to cover large urban or suburban residential neighborhoods with dense human populations.

Hypothetically, in some settings where focal early season enzootic WNV activity has been detected, early season adulticiding may be useful in interrupting virus transmission and lead to lower transmission rates later in the season. However, effective larval control of the principal enzootic mosquito vector is probably a more cost-effective way to interrupt early-season virus amplification.

#### **J. Evaluation of Adult Mosquito Control**

The following parameters should be periodically monitored during control operations:

1. Minimum requirements:
  - a) Pre- and post spray vector mosquito densities inside and outside control area using CO<sub>2</sub>-baited traps and gravid traps;
  - b) Vector mosquito infection rates pre- and post-spray inside and outside the control area; and
  - c) Weather conditions during application (temperature, wind speed, direction).
2. Desirable additions if capacity exists: population age structure of key mosquito species (*Cx. pipiens*)
3. In addition, both droplet size and flow rate should be documented for each piece of ULV application equipment:
4. During aerial application, GPS monitoring of spray track should be conducted if equipment is available on aircraft.

#### **K. Health Education, Public Information, and Human Behavior Change**

The goals of health education, public information, and behavior change programs are to inform the public about WNV, promote the adoption of preventive behaviors that reduce disease risk, and gain public support for control measures. Health education/public information includes use of print materials (posters, brochures, fact sheets), electronic information (Web sites), presentations (health experts or peers speaking to community groups), and the media.

Information alone is seldom sufficient to encourage people to adopt new behaviors or to change old practices. Programs should include strategies to facilitate protective actions and to address barriers that hinder preventive actions. Examples of programs that go beyond information include developing a community task force, interventions to improve access to window screening materials or repellents, and social marketing to reinforce preventive behaviors.

The following section covers key prevention messages, selected best practices, and research/program development priorities for promotion of personal and community measures to decrease risk of WNV infection. Public education and risk communication activities must be ramped up to respond to the degree of WNV risk in a community, as noted in Table 1.

##### **1. Key WNV Prevention Messages**

- a) Address the multiple levels at which prevention can occur: personal protection (use of repellent on skin and clothing, use of protective clothing, awareness of prime mosquito-biting hours); household protection (eliminating mosquito breeding sites, repairing/installing screens); and community protection (reporting dead birds, advocating for organized mosquito abatement, participating in community mobilization).
- b) Use of DEET-based repellents on skin and clothing is the backbone of personal protection. (For current recommendations, see [www.cdc.gov/ncidod/dvbid/westnile/qa/insect\\_repellent.htm](http://www.cdc.gov/ncidod/dvbid/westnile/qa/insect_repellent.htm).) Permethrin-based repellents should be promoted for use on clothing.
- c) Emphasize the feasibility of actions that can lower an individual's WNV risk through personal protection measures. Messages should acknowledge the seriousness of the disease but should not be fear-driven. Fear-driven messages may heighten the powerlessness many people express in dealing with emerging diseases.
- d) Recommendations to avoid being outdoors from dusk to dawn may conflict with neighborhood social patterns or practices of persons without air-conditioning or without other health programs seeking to increase physical activity. An alternative is to emphasize that the hours from dusk until dawn are prime mosquito-biting hours, and that protecting oneself through repellent use during these hours is important, with the option of remaining indoors.
- e) Communication about adulticiding: Public acceptance of emergency adult mosquito control is critical to its success, especially where mosquito control is unfamiliar or unpopular. Questions about the products being used, their safety, and their effects on the environment are common. Improved communication about surveillance and how decisions to adulticide are made may help residents weigh the risks and benefits of control. When possible, provide detailed information regarding the schedule for adulticiding through newspapers, radio, the Internet, or a recorded phone message
- f) Keep messages clear and consistent with the recommendations of coordinating agencies. Use plain language whenever possible, and adapt materials for lower-literacy and non-English speaking audiences.

## 2. Selected Best Practices

- a) Targeted prevention  
Audience members have different disease-related concerns and motivations for action. Proper message targeting permits better use of limited communication and prevention resources. The following are some audience groups that require specific targeting:
  - 1) *Persons over age 50*: While persons of any age can be infected with WNV, US surveillance data indicate that persons over age 50 are at higher risk for severe disease and death due to WNV infection.  
  
Collaborate with organizations that have an established relationship with mature adults, such as the AARP, senior centers, or programs for adult learners. Include images of older adults in your promotional material. Identify activities in your area where older adults may be exposed to



mosquito bites (e.g. jogging, golf, gardening).

- 2) *Persons with outdoor exposure:* While conclusive data are lacking, it is reasonable to infer that persons engaged in extensive outdoor work or recreational activities are at greater risk of being bitten by WNV-infected mosquitoes. Develop opportunities to inform people engaged in outdoor activities about WNV. Encourage use of repellent and protective clothing, particularly if outdoors during evening, night, or early morning hours. Local spokespersons (e.g., union officials, job-site supervisors, golf pros, gardening experts) may be useful collaborators.
- 3) *Homeless persons:* Extensive outdoor exposure and limited financial resources in this group present special challenges. Application of repellents with DEET or permethrin to clothing may be most appropriate for this population. Work with social service groups in your area to reach this population segment.
- 4) *Persons who live in residences lacking window screens:* The absence of intact window/door screens is a likely risk factor for exposure to mosquito bites. Focus attention on the need to repair screens and resources to do so. Partner with community organizations that can assist elderly persons or others with financial or physical barriers to screen installation or repair.

b) Partnerships with media and the community

Cultivate relationships with the media. Obtain media training for at least one member of your staff, and designate that individual as the organization's spokesperson. Develop clear press releases and an efficient system to answer press inquiries.

Develop partnerships with agencies/organizations that have relationships with populations at higher risk (such as persons over 50) or are otherwise recognized as community leaders (e.g., churches, service groups). Working through sources trusted by the target audience can heighten the credibility of and attention to messages. Partnerships with businesses that sell materials to fix or install window screens or that sell insect repellent may be useful in some settings.

c) Community mobilization and community outreach

Community mobilization can further education and behavior change goals. To counter any idea that health departments/mosquito control programs are able to control WNV alone, develop community ownership for prevention activities. A community task force that includes civic, business, health, and environmental concerns can be valuable in achieving buy-in from various segments of society and in developing a common message. Community mobilization activities can include clean-up days to get rid of mosquito breeding sites.

Community outreach involves presenting messages in person, in addition to media and educational materials, and incorporating citizens in prevention activities. Hearing the message of personal prevention from community leaders can validate the importance of the disease. Health promotion events reinforce the importance of prevention in a community setting.

### 3. Research and Program Development Priorities

#### a) Audience research

Attitudes toward arboviral disease prevention vary considerably by region. Previous experience with nuisance mosquitoes and mosquito control will affect the acceptability of prevention efforts. Audience research can identify local attitudes, motivations, barriers to prevention, and opportunities to promote desired behaviors.

Audience research should ideally combine qualitative and quantitative efforts. Surveys assessing knowledge, attitude, and practice levels in the target population can be very helpful, especially in evaluation, though they are a substantial undertaking. Qualitative research techniques, such as interviews and focus groups, can yield valuable data, and are more adaptable to resource levels. Expertise to undertake such efforts may be available from other divisions within a health department (e.g., chronic disease programs, maternal and child health).

Pretesting of educational materials is an important step to ensure the usability of materials by the intended audience. Pretesting does not always have to involve considerable time or expense; simply having representatives of the intended audience review materials before printing will be useful.

#### b) Evaluation

Outcome evaluation should be conducted whenever possible to measure the efficacy of the intervention in achieving protective behaviors (e.g., frequency of repellent use, presence of household mosquito breeding sites). Outcome measurement requires extensive effort and must be planned from the outset of a program.

#### c) Social marketing and risk communication

The goal of social marketing is to achieve specific behaviors, using the concepts of product, price, place, and promotion. Use of social marketing approaches can help programs plan to achieve specific behavior change goals.

Risk communication is already used by many health departments, and can be useful in refining communication messages for WNV, especially as the disease becomes endemic in new areas, and in discussing community control. Risk communication can help people analyze the choices that are available to them and to their community.

### 4. Resources

The CDC Web site ([www.cdc.gov/westnil](http://www.cdc.gov/westnil)) is updated frequently to reflect new findings and recommendations. Materials on the CDC Web site are generally in the public domain, and serve as a resource for state and local health departments and other organizations.

CDC staff can provide technical assistance in the development of audience research

and strategies for public education and community outreach. Contact CDC/Division of Vector-Borne Infectious Diseases' health communication staff at 970-221-6400. CDC can provide other communication planning resources, including CDCynergy ([www.cdc.gov/cdcynergy/](http://www.cdc.gov/cdcynergy/)), an interactive CD-ROM designed to help systematically plan health communication programs.

Other organizations that can provide useful information are the American Mosquito Control Association ([www.mosquito.org/](http://www.mosquito.org/)) and the National Pesticide Information Center (NPIC) ([npic.orst.edu](http://npic.orst.edu)), a program of EPA and Oregon State University concerning pesticides and repellents. They can be contacted at 1-800-858-7378.

#### **L. Legislation**

In addition to statutes permitting legal action to abate mosquito-related public health nuisances, legislation must be in place to allow creation of and provide funding for municipally-based integrated mosquito management programs. Local jurisdictions can contact state mosquito control associations to provide examples of enabling legislation.

#### **M. Guidelines for a Phased Response to WNV Surveillance Data**

The principal goal is to minimize the health impact of the WNV in humans, as well as in domestic and zoo animals. Given the limited understanding of the ecology and epidemiology of WNV in the U.S., the low incidence of arboviral encephalitis, and the limitations of prevention methods, prevention and control measures, regardless of intensity, may not prevent all WNV infections in humans.

The recommended response levels for the prevention and control of WNV should augment, but not replace, long-standing mosquito control efforts by established programs. These programs often have two objectives: 1) to control nuisance mosquitoes, and 2) to control vector mosquitoes that can transmit pathogens. Nuisance mosquito control often has different objectives than vector control, and the target mosquito species may also differ. Established mosquito control programs often have long-standing experience with the surveillance and control of indigenous neurotropic arboviruses such as SLE virus. These programs have established thresholds for response based on historical data. Long-standing experience with WNV does not exist in the U.S.

These guidelines for the prevention and control of WNV should be interpreted according to the following considerations:

1. All states should prepare for WNV activity. Given the extensive geographic spread of WNV since 1999, its occurrence in many different habitats and ecosystems in the Old World, its expansion into numerous habitat types in the Western Hemisphere, and the fact that SLE virus, a related flavivirus, is widespread in the U.S., there appear to be no barriers to the spread of WNV throughout the U. S. At a minimum, a plan for the surveillance, prevention, and control of WNV should be developed at the state and local levels.
2. Measures of the intensity of WNV epizootic in an area should be considered when determining the level of the public health response. Accumulating data analyses

indicate that intensity of epizootic WNV activity as measured by avian mortality and mosquito infection rates are good indicators of subsequently increased human infection risk. Data from NYC indicate that human WNV disease cases were more likely to occur in counties that had experienced more than 0.1 dead crow reports per square mile per week. In the Staten Island outbreak of 2000, the density exceeded 1.5 dead crow reports per square mile per week. Also, analysis of 2001 and 2002 surveillance data indicate that counties reporting WNV-infected dead birds early in the transmission season are more likely to report subsequent WNV disease cases in humans than are counties that do not report early WNV-infected dead birds. These observations should be interpreted as a guide rather than an absolute. Levels of epizootic activity that correlate with increased human risk will vary by region.

3. Flexibility is required when implementing the guidelines. Knowledge gained from ongoing surveillance and research could change the phased response recommendations. Specific and detailed recommendations that will fit all possible scenarios are not possible, particularly at a local level. Therefore, public health action should depend on interpreting the best available surveillance data in an area, in light of these general guidelines. In addition, the following factors should be considered when translating these guidelines into a plan of action:
  - a) Current weather and predicted climate anomalies;
  - b) Quality, availability, and timeliness of surveillance data;
  - c) Feasibility of the planned prevention and control activities, given existing budgets and infrastructure;
  - d) Public acceptance of the planned prevention and control strategies;
  - e) Expected future duration of WNV transmission (surveillance events earlier in the transmission season will generally have greater significance); and
  - f) Other ongoing mosquito control activities, such as nuisance mosquito control or vector mosquito control for the established arboviral encephalitis viruses.

The recommended phased response to WNV surveillance data is shown in Table 1. Local and regional characteristics may alter the risk level at which specific actions must be taken.

Table 1. Suggested Guidelines for Phased Response to WNV Surveillance Data

Risk category	Probability of human outbreak	Definition	Recommended response*
0	None	Off-season; adult vectors inactive; climate unsuitable.	Develop WNV response plan. Secure surveillance and control resources necessary to enable emergency response. Initiate community outreach and public education programs. Conduct audience research to develop/ target education & community involvement. Contact community partners.
1	Remote	Spring, summer, or fall; areas anticipating WNV epizootic based on previous WNV activity in the region; no current surveillance findings indicating WNV epizootic activity in the area.	Response as in category 0, plus: conduct entomologic survey (inventory and map mosquito populations, monitor larval and adult mosquito density), Initiate source reduction; use larvicides at specific sources identified by entomologic survey and targeted at likely amplifying and bridge vector species; Maintain avian mortality, vector and virus surveillance; Expand community outreach and public education programs focused on risk potential and personal protection, and emphasizing residential source reduction; Maintain surveillance (avian mortality, mosquito density /IR, human encephalitis/meningitis and equine illness).
2	Low	Summer, or fall; areas with limited or sporadic WNV epizootic activity in birds and/or mosquitoes. No positives prior to August.	Response as in category 1, plus: increase larval control, source reduction, and public education emphasizing personal protection measures, particularly among the elderly. Enhance human surveillance and activities to further quantify epizootic activity (e.g., mosquito trapping and testing). Implement adulticide applications if vector populations exceed locally established threshold levels, emphasizing areas where surveillance indicates potential for human risk to increase.
3	Moderate	Spring, summer, or fall; areas with initial confirmation of epizootic WNV in birds before August; a horse and/or a human case, or sustained WNV activity in birds and/or mosquitoes.	Response as in category 2, plus: intensify adult mosquito control in areas where surveillance indicates human risk, Initiate adult mosquito control if not already in progress, Initiate visible activities in community to increase attention to WNV transmission risk (speaker, social marketing efforts, community mobilization for source reduction, etc.), Work with collaborators to reduce risks to elderly (e.g., screen repair).
4	High	Spring, summer, or fall; quantitative measures indicating WNV epizootic activity at a level suggesting high risk of human infection (e.g., high dead bird densities In early summer, sustained high mosquito infection rates, multiple positive mosquito species, horse or mammal cases indicating escalating epizootic transmission, or a human case and high levels of epizootic activity). Areas with early season positive surveillance indicators where WN epidemic activity has occurred in the past.	Response as in category 3, plus: Expand public information program to include TV, radio, and newspapers (use of repellents, personal protection, continued source reduction, risk communication about adult mosquito control), Increase visibility of public messages, engage key local partners (e.g., government officials, religious leaders) to speak about WNV ; intensify and expand active surveillance for human cases; intensify adult mosquito control program, repeating applications in areas of high risk or human cases.

5	Outbreak in progress	Multiple confirmed cases in humans; Conditions favoring continued transmission to humans (e.g., persistent high infection rate in mosquitoes, continued avian mortality due to WNV)	Response as in category 4, plus: Intensify emergency adult mosquito control program repeating applications as necessary to achieve adequate control. Enhance risk communication about adult mosquito control. Monitor efficacy of spraying on target mosquito populations. If outbreak is widespread and covers multiple jurisdictions, consider a coordinated widespread aerial adulticide application; emphasize urgency of personal protection through community leaders and media, and emphasize use of repellent at visible public events.
---	----------------------	---	---

- 
- Local and regional characteristics may alter the risk level at which specific actions must be taken.

## **IV. HEALTH DEPARTMENT INFRASTRUCTURE**

### **State and Local Health Departments**

In the 48 contiguous United States, state and local health departments should have a functional arbovirus surveillance and response unit, staffed by well-trained personnel who have adequate data-processing resources, appropriate laboratory facilities, and an adequate operating budget. The size and complexity of these units will vary by jurisdiction, depending on both the risk of arboviral transmission in the area and available resources. A functional arbovirus surveillance unit at the state level should be considered an essential component of any emerging infectious diseases program. Local health department expertise and capabilities should be supported in a manner that complements statewide programmatic goals.

#### ***A. Staffing and Personnel***

Ideally, arboviral surveillance involves epidemiologists, virologists, medical entomologists, vertebrate biologists, veterinarians, laboratory staff, environmental toxicologists, public affairs personnel, and data managers. In a particular jurisdiction, the combination of personnel needed to conduct arboviral surveillance will depend on the importance of arboviral diseases in the area and on resources. Many health departments experience a chronic shortage or complete absence of medical entomologists and expertise in wildlife pathobiology. Addressing these deficiencies should be a high priority. In the event of an arboviral disease outbreak, local health departments will likely require significant surge capacity to ensure an adequate public health response. Contingency planning to identify resources to assist with the enhanced surveillance, laboratory, environmental, and public health needs should be identified ahead of time.

#### ***B. Training and Consultation***

Opportunities exist at federal and state agencies for appropriate training of and consultation with laboratorians, medical entomologists, epidemiologists, vertebrate biologists, and others involved in arbovirus surveillance.

#### ***C. Laboratory Capacity***

The infrastructure of arbovirus laboratories in the U.S. has deteriorated significantly in recent decades, not only in terms of the total number of functional laboratories and overall capacity, but also in terms of the staffing, physical plant, and financial support of many remaining laboratories. This is a problem of national scope and significance, the solution for which will require leadership at all levels of government.

##### **1. Testing for West Nile Virus (WNV) Infections**

In the wake of the introduction of WNV into the Western Hemisphere, it is important to distinguish between increasing short-term and long-term laboratory capacity. The latter is preferred and should be emphasized over the former. Laboratories with an existing capability for arbovirus serology should consider adding serologic screening tests for WNV to their repertoire. For serologic screening of patients and mosquito pools, arrangements can be made with CDC to transfer existing technology and reagents, and to obtain appropriate training. Samples giving positive or equivocal

screening results should be confirmed by CDC or another laboratory capable of definitive testing. For selected laboratories, similar technology transfer arrangements can be made with regard to RT-PCR primers for use in the testing of tissues and mosquito pools. In the wake of the recent epidemic of WN encephalitis in the Northeast, it is important that programs continue to routinely test for other arboviruses historically active in their area, such as St. Louis encephalitis, eastern equine encephalitis, western encephalitis, and La Crosse viruses, as well as for other causes of acute encephalitis.

#### **D. Developing Local Public Health Agency Infrastructure**

The function of local public health agencies is assessment, assurance, and policy development to promote and protect the health of the public. As part of this function, local public health agencies are responsible for preventive activities to reduce the risk of WNV infection to individuals in their jurisdictions. This responsibility includes educating communities about reducing mosquito breeding sites and taking personal protective measures. Local public health agencies also must have the capacity to assess human risk by gathering surveillance data or having access to surveillance data gathered on a district, regional, or statewide basis. These local public health agencies are important to formulating local recommendations on the indications and decisions concerning mosquito adulticiding. Education of and communication with the public, and maintenance of local media contacts are generally primary functions of the local public health agency. Included in this responsibility is communicating risk regarding the use of pesticides.

The following infrastructure and functional capacities fall within the province of local public health agencies. Where these are not directly provided, access to these capacities is to be ensured).

1. Risk assessment based on surveillance data (including mosquito, bird, and human data). Surveillance data may also include reports from individuals or healthcare providers indicating possible adverse health effects from pesticide use.
2. Health education regarding personal protection, reduction of mosquito breeding sites and minimum health risks posed by approved pesticides applied according to the label.<sup>73,74</sup>
3. Communication with the media.
4. Development of a preventive plan including education, mosquito source reduction, and larviciding.
5. Public response capability, particularly when surges of public inquiries arise. This may include the use of telephone hotlines and Internet Web sites.
6. Training of staff.
7. Coordination with state and federal agencies.
8. Local coordination by formulation of a task force with organizations such as departments of public works, offices of public affairs, city/county building management, departments of parks and recreation, departments of planning and zoning, property or building inspection services, police, public schools, colleges and



universities, nonprofit and grassroots organizations, businesses, zoos, animal/vector control, local mosquito control districts, emergency medical services, hospitals, poison control centers, departments of game and inland fisheries, departments of environmental quality, emergency, management agencies, etc.

## **V. INTERJURISDICTIONAL DATA SHARING AND NATIONAL REPORTING OF HUMAN CASES**

The public and animal health response to West Nile virus (WNV) epidemics/epizootics involves all levels of government, including the federal governments of the U.S. and neighboring countries, and the Pan American Health Organization. In addition, multiple government agencies at each level are often involved. Rapid, efficient, secure, and coordinated systems are needed to allow the sharing of human and ecologic data between these multiple agencies to support long-term surveillance activities, and to support activities that are part of the rapid outbreak response.

During an epidemic involving multiple jurisdictions, CDC staff and other authorized persons will use Epi-X, a CDC-sponsored, Web-based system for secured electronic communication, or similar integrated communication systems, for rapid dissemination of information on public health events of public health significance.

### ***A. Human Epidemiological, Clinical, and Laboratory Data Collection***

Patient confidentiality statutes vary among jurisdictions. Data can be shared between jurisdictions if recipients agree to adhere to the confidentiality statutes of the jurisdiction providing the data. Electronic databases should be appropriately secured by passwords to limit access and minimize opportunities for breaches in confidentiality or security.

### ***B. National Reporting of Human WNV Disease Cases***

#### **1. National Reporting of Human Cases of West Nile Meningoencephalitis (WNME)**

WNME is included in the list of nationally notifiable diseases maintained by the Council of State and Territorial Epidemiologists (CSTE) in consultation with CDC. CDC has designated 10056 as a specific disease code ("EVENT" code) for use in reporting WNME cases via the National Electronic Telecommunications System for Surveillance (NETSS). For national reporting purposes, states should use the national surveillance case definition of arboviral encephalitis/meningitis for classifying cases as either confirmed or probable (see Appendix C). Until such time as ArboNET and NETSS are consolidated under the National Electronic Disease Surveillance System (NEDSS) standards, duplicate reporting of human cases of WNME to both ArboNET and NETSS will be encouraged.

#### **2. West Nile Fever (WNF)**

Although WNF is not included in the list of nationally notifiable diseases, states are encouraged to report WNF cases to CDC via ArboNET, using a CDC recommended case definition (see Appendix D). States may also choose to report WNF cases to NETSS using EVENT code 10049.

### ***C. Ecologic Data***

Many of the issues that apply to the interjurisdictional sharing of human data apply to the sharing of ecologic data as well, although key differences exist. For example, confidentiality is generally not an issue with nonhuman cases, particularly wild animals identified as part of a surveillance program. Maintaining confidentiality may be important for certain owned animals. Data standardization is a far more challenging issue

because of the relatively large number of species being studied. Specific needs include the following:

**1. Accurate Taxonomic Identification of Specimens**

Fully understanding the epidemiology and developing effective prevention and control strategies for WNV requires accurate identification of all animal species involved in the virus transmission and maintenance cycles. This is especially true for birds and mosquitoes.

**2. Unique Identifier (UID) Numbering System for Specimens**

A UID numbering system should be used in each jurisdiction (e.g., state, county, city, surveillance area). Such a system should distinguish readily between each major animal group reported (i.e., humans, birds, and mosquitoes), and encode the location of collection (county or town), date of collection (day/month/year), and a specimen-specific number.

**3. Durable Tagging System for Field-Collected Specimens**

Use appropriate labels containing complete specimen information on all samples (blood, tissues, or whole animals) so field specimen identification will not be lost during shipment to testing facilities.

## **VI. RESEARCH PRIORITIES**

The human and animal health implications of the introduction of West Nile virus (WNV) to the U.S. and to the Western Hemisphere continue to emerge. Many questions remain, the answers to which will require considerable research. A research agenda should be supported, with priority given to research questions whose answers can be directly applied to prevention and control.

### ***A. Current and Future Geographic Distribution of WNV***

To determine the geographic distribution of WNV in the Western Hemisphere, existing laboratory-based surveillance systems for WNV in human, birds, other selected animals, and mosquitoes should be enhanced, or new, active systems should be developed and implemented (see Section I).

### ***B. Bird Migration as a Mechanism of WNV Dispersal***

Experience in Europe and the Middle East suggests that WNV regularly is introduced to new geographic areas along bird migration routes. A better understanding of this potential is required for the Western Hemisphere. Studies should include the frequency and duration of chronic infections that will allow the long-range transport and recrudescence of viremias necessary to infect mosquitoes.

### ***C. Vector and Vertebrate Host Relationships and Range***

Relatively little is known about the vertebrate host and mosquito vector relationships of WNV in the U.S. and the Western Hemisphere. Effective prevention and control strategies will require targeting selected species involved in maintenance, epidemic/epizootic transmission cycles, or both. It is critical that the principal species and the range of these species be determined.

### ***D. Virus Persistence Mechanisms***

It is not known whether or how WNV will be maintained in the U.S. over the long term. Overwintering mechanisms in *Culex* and *Aedes* species should be investigated, as well as persistence and maintenance of the virus in ticks. Other possibilities that should be investigated include the duration of chronic infection and reactivation in birds or other animals, and the introduction of the virus by migratory birds.

### ***E. Mosquito Biology, Behavior, Vector Competence, Surveillance, and Control***

It is critical that a better understanding is gained of the principal mosquito vectors involved in maintenance, bridge (from enzootic to peridomestic), and epidemic/epizootic transmission. Different vector species may be important in different geographic or ecologic regions. Understanding their biology and behavior will allow for more effective surveillance and development of targeted control methods.

### ***F. Development and Evaluation of Prevention Strategies***

Effective prevention and control of WNV transmission will require evaluation of the efficacy of current control methods and research on new and innovative control strategies for the principal mosquito vectors. Ultimately, prevention strategies must be integrated and use a variety of approaches to control mosquitoes and reduce the risk of transmission. Research should also be conducted to better define target areas for mosquito control in response to documented WNV activity in an area.

A very long-term goal is the identification and implementation of new, natural compounds to repel and control mosquito vectors of disease. With efforts to decertify current pesticides, new compounds will be needed in the fight against vector-borne diseases.

Much effort has been expended to increase public awareness of the WNV threat and of the actions needed to reduce exposure to infected mosquitoes. These actions include using mosquito repellents, reducing periresidential mosquito breeding sites, and wearing protective clothing when entering mosquito-infested areas. The success of these public information campaigns has not been formally evaluated using scientific instruments such as knowledge and behavior surveys. The cost of such campaigns is high, so formal attempts to assess their success are needed.

#### ***G. Laboratory Diagnosis***

Surveillance for WNV will continue to require accurate laboratory diagnostic tests. Ideally, these tests will be simple and inexpensive, and will distinguish between WNV and other flaviviruses such as the SLE, dengue, and yellow fever viruses. Virus-specific tests for IgM or IgG antibody will be required for humans, various species of birds, horses, and other mammals. Sensitive viral detection methods will be required for both human and animal tissues as well as for mosquito pools.

#### ***H. Clinical Spectrum of Disease and Long-Term Prognosis in Humans***

A better understanding of the spectrum of illness caused by WNV infection in humans is needed, including the long-term consequences of acute infection of the central nervous system. In addition to the severe end of the clinical spectrum (viral encephalitis), it is important to know the degree to which mild viral syndromes occur and whether these patients have any unique clinical presentations that may be characteristic or even pathognomonic. It is also important to know whether they have viremia and, if so, its magnitude and duration. Effective clinical management of severe disease will require detailed clinical studies of confirmed human cases of WNV infection.

#### ***I. Risk Factor Studies***

Data on the risk factors associated with human and animal infection with WNV are required to develop more effective prevention strategies, particularly when educating the public to take specific prevention measures to reduce exposure to infection.

#### ***J. Detailed Clinical Descriptions and Outcome in Human Cases***

Larger and more detailed case series, as well as studies of short- and long-term outcomes, are needed to better understand the clinical features, clinical course, and public health impact of WNV disease in humans. A suggested framework for collecting standardized "extended" clinical variables is included in Appendix E.

#### ***K. Viral Pathogenesis***

Little is known of the pathogenesis of WNV in humans or other animals. Research is needed to better understand the organ systems affected, the mechanism of central nervous system (CNS) infection, and the role of virus strain in pathogenesis.

#### ***L. Genetic Relationships and Molecular Basis of Virulence***

Only since 1996 has WNV been associated with significant numbers of severe disease cases and fatalities in humans. It is important to better understand whether genetic changes in WN viruses influence their phenotypic expression (i.e., host and vector

range, clinical expression in various hosts, and epidemic potential). This will require detailed studies of the genome of WN virus strains isolated from different epidemics in various geographic areas.

**M. Vaccine Development for Animals and Humans**

Ultimately, the most effective prevention strategy may be vaccination. It is important to support research on the development of both human and equine vaccines.

**N. Antiviral Therapy for West Nile Virus and Other Flaviviruses**

To date, none of the available antiviral agents are effective against flaviviruses, including WNV. Research in this area is critical to effective management of severe disease in humans.

**O. The Economic Cost of the WNV Epidemic/Epizootic**

It is important to estimate the total economic cost of the epidemic/epizootic. These data will help set priorities for capacity building and prevention programs.

**P. WNV Impact on Wildlife**

WNV has the potential to greatly impact the wildlife populations in the Western Hemisphere. This is especially true for birds, in many of which the infection appears to have high mortality rates (i.e., Corvidae). Research is needed to analyze and define this impact to determine if the development of new epizootic intervention strategies is needed. Research is also needed to determine what long-term effects WNV infection may have on its animal hosts.

**Q. Investigate Alternate Modes of WNV Transmission to Humans**

Four new modes of WNV transmission to humans were identified in 2002: blood transfusion, tissue transplantation, transplacental transfer, and breast-feeding. New modes of transmission should be investigated to determine the impact they have on human infection and to develop effective approaches for prevention and control of WNV infection by these routes.

## **Appendix A - National WNV Surveillance System**

### ***Objectives:***

The objectives of the national West Nile virus (WNV) surveillance system are to:

- Monitor the geographic and temporal spread of WNV in the U.S.
- Develop national public health strategies for WNV surveillance, prevention, and control.
- Develop a more complete regional picture of the geographic distribution and incidence of the other clinically important arboviruses in the U.S.
- Provide national and regional information to public health officials, elected government officials, and the public.
- Evaluate the use of cooperative agreement funds and the need for additional resources.

### ***Scope:***

Coordinated, multi-state surveillance of WNV infections in humans and animals has been repeatedly identified as a high priority by states affected by WNV in 1999-2002. All states conducting surveillance for WNV and other arboviruses are encouraged to participate in ArboNET, a CDC-coordinated program to collect these surveillance data. While the components of WNV surveillance systems employed in individual jurisdictions will vary, national WNV surveillance should, at a minimum, focus on collection of data from:

- Mosquito surveillance
- Avian (dead bird) surveillance
- Equine surveillance
- Human surveillance

In addition to data from states, data from commercial laboratories will be sought. CDC will 1) formally notify all such laboratories of the need to report any positive laboratory results to the appropriate state or local health department who, in turn, will notify CDC; 2) provide them with a list of state health department contact persons; 3) periodically contact them to encourage reporting; and 4) remind them of the need to have all positive screening tests for arboviral infections confirmed by state public health laboratories. In addition, CDC will provide a list of these commercial laboratories to its cooperative agreement partners, to facilitate their efforts to conduct active laboratory-based surveillance for arboviral infections.

### ***Categories of Data to be Collected:***

National surveillance will focus on the collection of two general categories of data:

- “Denominator” data

Definition: Weekly totals of dead birds (classified as either corvids or ‘others’) and mosquito pools (classified by species) collected and/or tested by a jurisdiction’s WNV surveillance system, stratified by county within a state. Because recent experience has demonstrated that the following categories of denominator data are of limited use in meeting national surveillance goals, as of 2003, CDC will discontinue the collection of totals of sentinel and free-ranging wild birds, horses, or other non-human mammals tested.

- “Numerator” data

Definition: Detailed information on individual mosquito pools, sentinel species, dead birds, and ill humans, horses, or other species with confirmed or suspected WNV infections, as determined by laboratory-confirmed or -probable test results.

**General Procedures:**

**Reporting “denominator” data:**

CDC will collect aggregate denominator data via a secure file upload system using a state-based database provided by CDC, continuous data entry into a database stored on a secured CDC web site, or importation of delimited records in a specified format. Denominator data variables are specified in Table 1. An appropriate submission schedule will be arranged by CDC with the jurisdictions submitting surveillance data via file uploading. In addition,

- CDC will distribute the necessary software and provide the adequate licenses that will allow regular secured file upload or continuous web-based data entry.
- CDC will accommodate state health departments with existing integrated data collection systems, e.g., by arranging for uploads of XML-formatted data.
- The data entry screens will be designed as a series of simple forms or tables.
- The system will accommodate updates and corrections of previously transmitted data by jurisdictions.
- Following the entry of a week’s data into the database at the state level, transmission of the data file to CDC will involve a minimal number of keystrokes. Security will be insured by use of the sender’s digital certificate. CDC will arrange for those who will be transmitting surveillance data to CDC to obtain digital certificates.
- Upon arrival at CDC, records from the specific reporting week of interest will automatically be captured and imported into a master database on the CDC fileserver and also transmitted to USGS in Reston, Virginia.
- Using these data, reports will be generated automatically each week. Maps will be generated by CDC and USGS and made available on the USGS web site. A basic set of dynamic maps and corresponding graphs and tables will be made available weekly. The CDC web site and Epi-X (or a similar secured communication network) will contain links to the relevant USGS web pages.



### **Reporting “numerator” data:**

CDC strongly encourages prompt (“real-time”) reporting of numerator data. CDC will collect such reports in a standardized manner to allow monitoring of regional and national trends, and facilitate prompt confirmatory testing when necessary. As the arbovirus transmission season progresses, the need for immediate reporting of certain data to CDC may diminish. For example, once numerous WNV-positive mosquito pools have been previously documented in a given geographic area, there may not be a compelling need to *immediately* report further findings. In addition, if at any time the volume of reporting becomes overwhelming, adoption of an alternative system may be necessary.

Numerator data variables that will be collected are specified in Table 2. WNV laboratory and surveillance case criteria are specified in Table 3.

Specified, line-listed numerator data may be submitted using one of three methods:

- Web-based data entry to a CDC server;
- Use of state-based, CDC-distributed, Microsoft Access-based data entry/management software (ArboNET) with continuous file upload to a CDC server; or
- Data messaging from a unique data collection system to a CDC server (e.g., in XML format).

All data entry will be done by the reporting jurisdiction and data is transmitted to a CDC server. After data entry and submission, numerator data will be available on the CDC Secure Data Network (SDN) so that authorized personnel from the reporting jurisdiction may “verify” (proofread, correct, and clear for publication) individual numerator data records in selected surveillance categories.

It is essential that each numerator data record include a unique identifier (UID) assigned by the reporting state agency. UIDs will be used by CDC staff to track and update individual numerator data records, and by states to verify records via the CDC SDN. The UID will not appear in output products for public release. Most jurisdictions already have systems in place for generating UIDs, and they should continue to use them. CDC’s databases will accommodate numeric or alphanumeric UIDs up to 25 characters long. Jurisdictions are encouraged to begin their UIDs with their state’s 2-letter postal code (or “NYC” for New York City).

The issue of numerator data records associated with laboratory-probable results deserves special mention. Although CDC encourages confirmation of all laboratory-probable results, it is realized that under some circumstances some states may choose not to do so, depending on the epidemiologic situation, laboratory capacity, and volume. For example, during a known WN viral epizootic, a state may decide that a crow brain associated with a single positive result for WN viral RNA by RT-PCR will undergo no further testing. Although this bird is a laboratory-probable case (see table below), the jurisdiction may decide to upload that bird’s numerator data record to CDC and subsequently authorize CDC to release it publicly. In contrast, a jurisdiction may opt to delay the release of such results to the public until they have been laboratory-confirmed. CDC will rely on individual jurisdictions to decide when to authorize the public release of numerator data records based on *laboratory-probable* results.

CDC will not publicize numerator data records associated with laboratory-equivocal results.

In terms of human surveillance, the national surveillance case definition of arboviral encephalitis/meningitis includes two official case-status categories: confirmed and probable (Table 3). For national arboviral encephalitis surveillance, CDC has traditionally combined records in these two categories for its annual summary reports, and will continue this practice within the WNV surveillance system. States are encouraged to promptly report both laboratory-confirmed and laboratory-probable human WN encephalitis cases as numerator data records.

CDC encourages the reporting of human WN viral illnesses other than WNME (e.g., WNF, acute flaccid paralysis, other clinical syndrome, or unspecified). To determine case status (confirmed or probable) for reporting purposes, refer to the national surveillance case definition of arboviral encephalitis/meningitis (Appendix C) and the CDC-recommended surveillance case definition for WNF (Appendix D). A working case definition for WNME in equines is shown in Appendix B.

### ***Arboviruses other than WNV:***

It is anticipated that enhanced WNV surveillance will result in increased recognition of other domestic arboviral activity, including eastern equine encephalitis (EEE), western equine encephalitis (WEE), SLE, La Crosse (LAC), and Powassan (POW) virus activity. Surveillance numerator (laboratory-positive) data regarding these viruses may be reported to CDC/DVBID via ArboNET, telephone, FAX, or e-mail.

### **Data Security Issues:**

General principles:

- State and local health authorities will retain control of the timing of data release.
- As of 2003, reporting agencies will electronically report to ArboNET all categories of surveillance data, including human numerator data. For non-human data, agencies will verify accuracy and readiness for public release prior to submission. Upon the electronic submission of non-human data to CDC, these reports will be considered verified and publishable. With the 2003 version of ArboNET, human data will be automatically verified upon entry, and the reporting agency has the option to unverify the data via electronic checkbox. CDC will not publicly release unverified human case reports.
- Personal identifying or localizing (more specific than county) information will not be released.

Specific issues:

- To report data via secure file upload to the CDC fileserver or to enter data directly onto a secured web site, states will utilize the CDC SDN, which provides data encryption for transmission via the Internet. To use the SDN, users must obtain and install a digital certificate from the CDC certificate server. This allows for unique identification of the computer/browser that is accessing a secure web site.

- To obtain a digital certificate and be approved to use the SDN, the digital certificate authority at CDC/DVBID must approve the request and forward it to CDC/Atlanta. CDC requests that a maximum of 3 persons from each state be designated to receive digital certification. These should include those who will transmit data to CDC, as well as those who will verify data on the SDN.

### **Summary Reports to be Produced by CDC and USGS:**

A working list of basic summary reports is shown in Table 4. The exact list and formats of these reports remain to be determined, and this should be viewed as a dynamic process. Modifications, additions, and deletions may take place over time, as dictated by feedback, experience, technical issues, and events.

Using state-approved numerator and denominator data, reports will be generated weekly. Maps and tables will be generated by DVBID and by USGS. Maps and corresponding graphs and tables will be updated at least weekly on the USGS web site ([www.USGS.gov](http://www.USGS.gov)).

### **Communication Issues:**

- A dedicated telephone line (970-266-3592), electronic mailbox ([dvbid2@cdc.gov](mailto:dvbid2@cdc.gov)), and fax machine (970-266-3599) will be available at CDC/DVBID (in Fort Collins, Colorado) 24 hours/day for reporting numerator data or other urgent WNV-related business. During nights and weekends, calls to the dedicated phone line will be forwarded to the cellular phone of an on-call CDC/DVBID staff scientist. *Because of potential delays in the receipt and reading of email and fax messages, in general please use the telephone for time-sensitive business.*
- In addition to periodic conference calls between CDC, cooperating states, and other federal agencies, Epi-X and the WNV Information Exchange (WNVIX, part of the Epi-X Forum) will be available to participating jurisdictions and agencies using the CDC SDN. For further information, contact the CDC/DVBID ArboNET staff at 970.221.6400 or send electronic mail to [dvbid2@cdc.gov](mailto:dvbid2@cdc.gov).

Submission of Laboratory Specimens to CDC for WNV Testing:

See Table 5.

Table 1. *Denominator* Data Variable List

(Note: As of 2003, *denominator* data will no longer be collected in the following categories: sentinel animals, seroprevalence in free-ranging birds, and ill equines or humans.)

**I. Avian mortality:** (Includes ill or dead birds, except for sentinels.)

- Year
- MMWR week that bird collected (“MMWR week collected”)  
(Note: “MMWR week collected” corresponds to the earliest date associated with a specimen. Preferably, this should be MMWR week that corresponds to *the date that the bird was reported by the public*. But, if a date of report is not available, use the MMWR week that corresponds to the *date that the specimen was collected in the field*. This “MMWR week collected” should remain associated with this specimen **throughout testing**.)
- County
- State
- Number of reported corvids by “MMWR week collected” and by county (Data source: State, county or township WNV surveillance coordinators through the state to CDC)
- Number of corvids **tested** by “MMWR week collected” and by county (Data source: Testing laboratories through state)
- Number of other reported birds by “MMWR week collected” and by county (Data source: Jurisdictional WNV surveillance coordinators to CDC via state or municipal health departments)
- Number of other birds **tested** by “MMWR week collected” and by county (Data source: Testing laboratories through state)

Note: Laboratory-positive” results are reported through the *numerator* system by the testing facility/agency. In this report, the date of reporting/sighting or field collection is routinely obtained. By definition, each *numerator* data record of a WNV-positive dead bird should also be included within an aggregated denominator data record.)

**II. Mosquito collections:**

- Year
- MMWR week of collection  
(Note: This is the MMWR week that corresponds to the date of field collection. This date should remain associated with this specimen **throughout testing**.)
- County
- State
- Species of mosquito
- Number of mosquitoes collected by MMWR week of collection, by county, and by species (Data source: Jurisdictional WNV surveillance coordinators to CDC via state or municipal health departments)
- Number of mosquitoes **tested** by MMWR week of collection, by county, and by species (Data source: Testing laboratories through state).

(Note: Laboratory-positive results are reported through the *numerator* system by the testing facility/agency. In this report, the date of field collection is routinely obtained. By definition, each *numerator* data record of a WNV-positive mosquito pool should also be included within an aggregated denominator data record.)

Table 2. *Numerator* data variables

***Mosquito surveillance*** – state, county, pool UID, date of mosquito collection, week of collection, species, arbovirus, case status

***Sentinel species surveillance*** - State, county, group UID, date of serum collection, week of serum collection, species, arbovirus, case status

***Avian mortality surveillance*** – state, county, bird UID, week bird found collected, date bird collected, species (including “captive species”), arbovirus, case status

***Avian seroprevalence surveillance*** – state, county, bird UID, week bird trapped & bled, date bird trapped & bled, species, arbovirus, case status

***Veterinary (non-avian) surveillance*** – state, county, animal UID, week of illness onset, date of illness onset, species (canine, equine, feline, bat, squirrel, rabbit, raccoon, or other species), arbovirus, case status.

***Human surveillance*** – state, county, patient UID, week of illness onset, date of illness onset, imported from, arbovirus, case status, age, age unit, birthdate, sex, race, ethnicity, clinical syndrome, fatality, date of death, lab acquired, non-lab acquired, blood donor, blood recipient. organ donor, organ transplant recipient, breast fed infant at time of illness, potential in-utero infection, pregnant at time of illness

Table 3. WNV Laboratory and Surveillance Case Criteria

Laboratory case definitions:

Surveillance Type	Laboratory-confirmed WNV infection	Laboratory-probable WNV infection*
Mosquito	<ul style="list-style-type: none"> <li>• WNV isolation (identity of virus established by at least two of the following techniques:</li> <li>• Positive RT-PCR test for WN viral RNA with validation by 1) repeated positive test using different primers, 2) positive PCR result using another system (e.g., TaqMan), or 3) virus isolation.</li> <li>• Detection of WN viral antigen (e.g., IFA, EIA, VecTest™) validated by inhibition test (for ELISA), RT-PCR, or virus isolation</li> </ul>	<ul style="list-style-type: none"> <li>• Positive RT-PCR test for WN viral RNA in a single test</li> <li>• Antigen detection not validated by another procedure</li> </ul>
Sentinel species	<ul style="list-style-type: none"> <li>• WNV isolation, RNA detection, or antigen detection as described for mosquitoes,</li> <li>• Seroconversion to WNV in serially collected serum specimens, by plaque-reduction neutralization**</li> <li>• Detection of IgM antibody to WNV, validated by demonstration of neutralizing antibody to WNV**</li> </ul>	<ul style="list-style-type: none"> <li>• Detection of IgM antibody to WNV</li> <li>• Seroconversion to WNV in serially collected serum specimens, strongly reactive by EIA or IFA</li> </ul>
Avian mortality	<ul style="list-style-type: none"> <li>• WNV isolation, RNA detection, or antigen detection as described for mosquitoes, ,</li> </ul>	<ul style="list-style-type: none"> <li>• Positive RT-PCR test for WN viral RNA in a single test</li> <li>• Antigen detection not validated by another procedure</li> </ul>

Surveillance Type	Laboratory-confirmed WNV infection	Laboratory-probable WNV infection*
Veterinary (non-avian)	<ul style="list-style-type: none"> <li>As for humans (see below)</li> </ul>	<ul style="list-style-type: none"> <li>As for humans (see below)</li> </ul>
Human	<ul style="list-style-type: none"> <li>See national surveillance case definitions (Appendices C and D)</li> </ul>	<ul style="list-style-type: none"> <li>See national surveillance case definitions (Appendices C and D)</li> </ul>

\* CDC strongly encourages attempts to confirm all laboratory-probable and -equivocal results. Further testing of laboratory-probable human specimens will depend on availability of confirmatory testing.

\*\* SLE virus infection should be ruled-out by cross-neutralization; criterion for PRNT positive is a 90% neutralization titer of at least 1:10, and 4-fold greater titer compared to other flaviviruses such as SLE.

Table 4. Working List of Basic Weekly Summary Reports to be Produced by CDC

*NOTE: The exact list and formats of these reports remain to be determined, and this should be viewed as a dynamic process. Modifications, additions, and deletions may occur over time, as dictated by feedback, experience, technical issues, and events.*

- A. National map: U.S. map with state boundaries reflecting cumulative data.
  - 1. Mosquito surveillance:
    - a. Map showing each state's counties as WNV-positive, WNV-negative, or blank (no data)
  - 2. Sentinel chicken surveillance:
    - a. Map showing each state's counties as WNV-positive or blank (no data)
  - 3. Avian morbidity/mortality surveillance:
    - a. Map showing each state's counties as WNV-positive, WNV-negative, or blank (no data)
  - 4. Veterinary (non-avian) surveillance:
    - a. Map showing each state's counties as WNV-positive (# cases) or blank (no data)
  - 5. Human surveillance:
    - a. Map showing each state's counties as WNV-positive (# cases) or blank (no data)
  
- B. State Maps: Selecting an individual state from the national map will produce a map of that state with its county boundaries indicating the positive specimens reported for that county and an accompanying table of cumulative positive specimens reported by county.
  - 1. Mosquito surveillance:
    - a. Map showing each county as WNV-positive with a count of positive specimens reported, WNV-negative, or blank (no data)
  - 2. Sentinel species surveillance:
    - a. Map showing each county as WNV-positive with a count of positive specimens reported or blank (no data) by sentinel species (e.g., horse, chicken)
  - 3. Avian mortality surveillance:
    - a. Map showing each county as WNV-positive with a count of positive specimens reported, WNV-negative, or blank (no data)
  - 4. Veterinary (non-avian) surveillance:
    - a. Map showing each county as WNV-positive with a count of positive specimens reported or blank (no data)
  - 5. Human surveillance:
    - a. Map showing each county as WNV-positive with a count of positive specimens reported or blank (no data)



Table 5. Instructions for Submitting Laboratory Specimens to CDC for WNV Testing

### **Arrangements for Testing:**

*Mosquito specimens:* Specimens will be accepted for confirmatory testing at CDC when requested by a state health department vector surveillance coordinator. For specimens considered by a state health department vector surveillance coordinator to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Roger Nasci, tel. 970-221-6432, RNasci@cdc.gov; if Dr. Nasci cannot be reached, please phone 970-266-3592.

*Sentinel chicken specimens:* Serum specimens will be accepted for confirmatory testing at CDC when requested by a state health department vector or vertebrate surveillance coordinator. For specimens considered by a state health department vector or vertebrate surveillance coordinator to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

*Avian morbidity/mortality specimens:* On a case-by-case basis, special arrangements can be made for CDC to conduct initial and/or confirmatory tests of tissue specimens (especially brain, heart, kidney, and spleen) from dead birds that cannot otherwise be tested in state health department laboratories or by the National Wildlife Health Center, USGS. For further information, please contact Dr. Nick Komar, tel. 970-221-6496, NKomar@cdc.gov; if Dr. Komar cannot be reached, please call 970-266-3592.

*Veterinary (non-avian) specimens:* Specimens will be accepted for confirmatory testing at CDC when requested by a state health department laboratory director. For routine testing of veterinary specimens, contact the state health department laboratory or the National Veterinary Services Laboratory, USDA, in Ames, IA (Tel. 515-663-7751), or another collaborating laboratory. For specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of that state's public health laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

*Human specimens:* Specimens will be accepted for confirmatory testing at CDC when requested by a state health department laboratory director. For specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

### **General Shipping Instructions:**

All shippers should adhere to International Air Transport Association regulations (<http://www.iata.org>).

Specimens should be shipped by overnight courier to arrive at CDC on Tuesday-Friday. *Always notify CDC staff in advance of an impending shipment* (tel. 970-221-6445; if no answer, phone 970-266-3592). Do not ship specimens on Friday unless special arrangements have been made.

**Shipping address:** CDC/DVBID  
CSU Foothills Campus/Rampart Road  
Fort Collins, CO 80521  
ATTENTION: Arbovirus Diagnostic Laboratory (tel. 970-221-6445)

**Shipping containers:** Use only durable containers. Seal specimen containers tightly. Wrap specimen containers in absorbent material and pack them into two different plastic containers to insure that any leakage is contained. Specimens for virus isolation must be sent on enough dry ice to insure that they remain frozen until receipt. Specimens for serologic testing can be shipped on gel-ice and need not remain frozen. Hand-carrying specimens is not recommended but if specimens are hand-carried, the above packing instructions are applicable.

### **Minimal Information to Accompany Specimens Shipped to CDC:**

See information in columns 2, 3, and 4 in Table 2. Please read carefully and supply all available information. Use CDC Form 5034 (the “DASH” form) Form 5034 is available electronically at: [http://www.cdc.gov/ncidod/dvbid/CDC\\_form5034.pdf](http://www.cdc.gov/ncidod/dvbid/CDC_form5034.pdf)

Tubes, cryovials, and other specimen containers should be clearly labeled with – at minimum – the specimen’s UID, patient’s name (human), state, date of onset, date of collection, and specimen type.

### **Special Collection, Shipping, and Handling Instructions:**

**Mosquitoes:** Ship on dry ice.

**Serum:** Store in externally threaded plastic tubes. Ship at least 0.5 mL per specimen. Whenever possible, acute and convalescent specimens should be shipped together. Ship fresh-frozen on dry ice (required for virus isolation) or refrigerated on wet ice (acceptable).

**CSF:** Store in externally threaded plastic tubes. Ship at least 1.0 mL per specimen. Ship fresh-frozen on dry ice (required for virus isolation) or refrigerated on wet ice (acceptable).

**Whole blood:** In general, send only if requested for virus isolation attempts in fatal cases (heart blood).

**Pregnancy-related specimens:** In possible cases of intrauterine arboviral infection, tissues collected at the time of delivery can be tested for evidence of infection. The following tissues should be shipped fresh-frozen on dry ice: cross-sections of umbilical cord, placental tissue (approximately 1 cm<sup>3</sup> per sample), cord serum and maternal serum (0.5 ml each), and colostrum or breast milk. For more information, please contact Dr. Dan O’Leary at (970) 266-3525 or [DOLeary@cdc.gov](mailto:DOLeary@cdc.gov).

**Autopsy specimens:** In suspected cases of arboviral encephalitis in which an autopsy is performed, **fresh-frozen** tissues can be tested, including brain (multiple areas of cortex,

midbrain, brainstem, and spinal cord), other solid organs (liver, spleen, pancreas, heart, kidney, etc.), CSF (collected from ventricles), and heart blood (for virus isolation attempts).

After consulting with Dr. Sherif Zaki or other CDC/Atlanta pathology staff members (tel. 404-639-3133), **tissue samples suspended in formalin** should be sent to:

Infectious Disease Pathology Activity  
DVRD/NCID/CDC  
Building 1, Room 2301  
1600 Clifton Road, N. E.  
Atlanta, GA 30333

*Veterinary (non-avian) tissues:* As for human specimens.

*Avian tissues:* Submit fresh-frozen brain, heart, kidney, and spleen samples.

## Appendix B – Surveillance Case Definition for WNV Infection in Equines

### Laboratory criteria for diagnosis

Compatible clinical signs<sup>[1]</sup> plus one or more of the following:

- Isolation of West Nile (WN) virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF) or other body fluid;<sup>[2]</sup> or
- Detection of IgM antibody against WN virus by IgM-capture ELISA in serum (at 1:400 or greater dilution) or cerebrospinal fluid (CSF) (at dilution 1:2 or greater dilution); or
- An associated 4-fold or greater change in IgG-capture ELISA or plaque-reduction neutralization test (PRNT) antibody titer to WN virus in appropriately timed,<sup>[3]</sup> paired serum specimens from an equid that is unvaccinated against WN virus; or
- Positive immunohistochemistry (IHC) for WN virus antigen in tissue.

### Case classification

**Probable:** compatible clinical signs occurring during a period when arboviral transmission is likely, and with the following supportive serology: 1) a single or stable (less than or equal to two-fold change) but elevated titer of WN virus-specific IgM-capture ELISA or neutralizing serum antibodies without knowledge of prior WN virus vaccination.

**Confirmed:** compatible clinical signs with laboratory-confirmed evidence of WN virus infection.

### Notes:

- [1] Clinical signs are associated with central and/or peripheral nervous system dysfunction. Most horses exhibit secondary CNS-derived neurological manifestations such as ataxia (including stumbling, staggering, wobbly gait, or incoordination) or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, altered mental status, blindness, lip droop/paralysis, teeth grinding. (Ostlund et al, Equine West Nile Encephalitis, United States, Emerging Infectious Diseases, Vol 7, No 4. Jul – Aug 2001) Fever is not a consistent finding.
- [2] Preferred diagnostic tissues from equids are brain or spinal cord; isolation of WN virus or detection of WN viral nucleic acid sequences in equine blood or CSF are infrequent. (Bunning et al, Experimental Infection of Horses with *West Nile virus*, Vol 8, No. 4. April 2002)
- [3] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least 14 days post-onset.

---

### Assumptions on which case definitions are based:

- IgM-capture ELISA testing may give nonspecific results; cross-reactions to closely related flaviviruses (e.g., St. Louis encephalitis virus) may occur. Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests

using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conduction cross-neutralization tests using an appropriate battery of closely related viruses.

- Vaccination refers to one or more doses of the current USDA-licensed inactivated WN virus vaccine.
- IgM antibody in equine serum is relatively short-lived (how long);(ref?) a positive IgM-capture ELISA means infection with WN virus or a closely related flavivirus has occurred, probably within the last three months. (personal communication **Eileen N. Ostlund, USDA**)
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WN virus; it is possible that clinical signs may be present in an equine before a serum PRNT is positive. (ref)
- Neutralizing antibody detected in serum by PRNT indicates past infection with WN virus or vaccination with WN virus vaccine; equines exposed to WN virus in prior years may test positive by PRNT.

## **Appendix C - National Surveillance Case Definition for Arboviral Encephalitis/Meningitis, 2001**

(available at <http://www.cdc.gov/epo/dphsi/casedef/encephalitiscurrent.htm>)

### **Encephalitis or Meningitis, Arboviral (includes California serogroup, Eastern equine, St. Louis, Western equine, West Nile, Powassan)**

#### **2001 Case Definition**

##### **Clinical description**

Arboviral infections may be asymptomatic or may result in illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes ranging from febrile headache to aseptic meningitis to encephalitis may occur, and these are usually indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis. Arboviral encephalitis is characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction (e.g., paresis or paralysis, cranial nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements).

##### **Laboratory criteria for diagnosis**

- Fourfold or greater change in virus-specific serum antibody titer, or
- Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, or
- Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA), or
- Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g., neutralization or hemagglutination inhibition).

##### **Case classification**

*Probable:* an encephalitis or meningitis case occurring during a period when arboviral transmission is likely, and with the following supportive serology: 1) a single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or 2) serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen.

*Confirmed:* an encephalitis or meningitis case that is laboratory confirmed.

##### **Comment**

Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected

against St. Louis encephalitis virus are not the result of an infection with WN (or dengue) virus, or vice versa, in areas where both of these viruses occur.

The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions. Reporting should be etiology-specific (see below; the six encephalitides/meningitides printed in bold are nationally reportable to CDC):

**St. Louis encephalitis/meningitis** (NETSS Event Code: 10051)

**West Nile encephalitis/meningitis** (NETSS Event Code: 10056)

**Powassan encephalitis/meningitis** (NETSS Event Code: 10057)

**Eastern equine encephalitis/meningitis** (NETSS Event Code: 10053)

**Western equine encephalitis/meningitis** (NETSS Event Code: 10052)

**California serogroup viral encephalitis/meningitis** (includes infections with the following viruses: La Crosse, Jamestown Canyon, snowshoe hare, trivittatus, Keystone, and California encephalitis viruses) (NETSS Event Code: 10054)

Other viral CNS infections transmitted by mosquitoes, ticks, or midges (e.g., Venezuelan equine encephalitis/meningitis [NETSS Event Code: 10055] and Cache Valley encephalitis/meningitis [NETSS Event Code: 10058])

## Appendix D - CDC-Recommended Surveillance Case Definition for WN Fever

### What is a CDC-Recommended Case Definition?

CDC-recommended surveillance case definitions are prepared for use by U.S. States and Territories interested in conducting public health surveillance for diseases or conditions that have not been designated nationally notifiable and have not been officially approved and sanctioned by the Council of State and Territorial Epidemiologists (CSTE). A CDC-recommended case definition may not be approved by CSTE in the future, unless CSTE and the CDC program with responsibility for prevention and control of the selected disease or condition both wish to seek broader and more formalized approval from both organizations.

### **CASE DEFINITION**

#### Case Description

A non-specific, self-limited, febrile illness caused by infection with WNV, a mosquito-borne flavivirus. Clinical disease generally occurs 2-6 days (range, 2-15 days) following the bite of an infected mosquito. Typical cases are characterized by the acute onset of fever, headache, arthralgias, myalgias, and fatigue. Maculopapular rash and lymphadenopathy generally are observed in less than 20% of cases. Illness typically lasts 2-7 days.

#### Case Classification

A clinically compatible illness, *plus*:

#### **Confirmed:**

- 1) Fourfold or greater change in WNV-specific serum antibody titer;
- 2) Isolation of WNV from or demonstration of specific WN viral antigen or genomic sequences in tissue, blood, CSF, or other bodily fluid; or
- 3) WNV-specific IgM antibodies demonstrated in serum by antibody-capture enzyme immunoassay and confirmed by demonstration of WNV-specific serum neutralizing antibodies in the same or a later specimen.

#### **Probable:**

- 1) WNV-specific serum IgM antibodies detected by antibody-capture enzyme immunoassay but with no available results of a confirmatory test for WNV-specific serum neutralizing antibodies in the same or a later specimen.

*(Note: Some WN fever cases progress to WN meningitis or encephalitis. Cases meeting the more restrictive case definition of WN encephalitis/meningitis should be reported as such and only once, using event code 10056 for "WN Encephalitis or Meningitis".)*



## Comment

The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions. Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to identify the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against WNV are not the result of an infection with St. Louis encephalitis or dengue virus, or vice versa. Because dengue fever and WN fever can be clinically indistinguishable, the importance of a recent travel history and appropriate serologic testing cannot be overemphasized. In some persons, WNV-specific serum IgM antibody can wane slowly and be detectable for more than one year following infection. Therefore, in areas where WNV has circulated in the recent past, the co-existence of WNV-specific IgM antibody and illness in a given case may be coincidental and unrelated. In those areas, the testing of serially collected serum specimens assumes added importance.

**Date case definition was developed:** October 2002

**Event Code:** 10049

**Source of the case definition:** National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases, Arbovirus Diseases Branch.

**Questions and comments about the case definition should be directed to the following CDC/ADB staff:**

Roy Campbell

Phone: (970) 221-6459

E-mail: [glc5@cdc.gov](mailto:glc5@cdc.gov)

Dan O'Leary

Phone: (970) 266-3525

E-mail: [dbo7@cdc.gov](mailto:dbo7@cdc.gov)

Tony Marfin

Phone: (970) 266-3521

E-mail: [aam0@cdc.gov](mailto:aam0@cdc.gov)

## Appendix E – Recommended Framework for Standardized “Extended” Clinical Variables in Studies of Human WNV Disease

Larger and more detailed case series, as well as studies of short- and long-term outcome, are needed to better understand the clinical features, clinical course, and public health impact of WNV disease in humans. A suggested framework for collecting standardized “extended” clinical variables is shown below. During 2003, CDC will work with its partners to populate this framework with specific questions in each category. The use of standardized questions will allow public health officials and other researchers to compare results more readily.

1. Epi core data (e.g., age, gender, residence location, race/ethnicity, type of West Nile virus illness, etc.) *[Note: These are already standard ArboNET variables.]*
2. Past medical history
3. Previous arboviral infections or vaccinations
4. Immunosuppressed conditions
5. New modes of transmission
6. Clinical presentation – neurology and initial symptoms
7. Clinical presentation – Standardized scale of neuro/physiologic function (e.g., APACHE, Glasgow Coma Scale, PRISM)
8. Clinical presentation – laboratory
9. Clinical presentation – WNV diagnostic studies
10. Clinical presentation – Special diagnostic studies (e.g., MRI, EEG, EMG, lumbar puncture)
11. Treatment (e.g., antivirals, steroids, anti-seizure medications, hyperventilation, interferon, intravenous immunoglobulin, plasmapheresis)
12. Clinical course (e.g., renal function, electrolyte balance, neurologic complications)
13. Morbidity (e.g., number of hospital-, ICU-, and ventilator-days, number and type of nosocomial infections, etc.) *[Note: Many of the morbidity parameters can be used in determining the costs of WNV disease.]*
14. Nosocomial infections
15. Discharge disposition
16. Neurologic and functional status at disposition, at 90 days post-discharge, and 180 days post-discharge
17. Mortality (e.g., cause of death, pathology findings, etc.)

## REFERENCES:

1. Centers for Disease Control and Prevention. Outbreak of West Nile-like viral encephalitis--New York, 1999. *MMWR Morb Mortal Wkly Rep* 1999;48:845-9.
2. Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *New Engl J Med* 2001;344:1807-14.
3. Anderson JF, Andreadis TG, Vossbrinck CR, Tirrell S, Wakem EM, French RA, et al. Isolation of West Nile virus from mosquitoes, crows, and a Cooper's hawk in Connecticut. *Science* 1999;286:2331-3.
4. Briese T, Jia XY, Huang C, Grady LJ, Lipkin WI. Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis. *Lancet* 1999;354:1261-2.
5. Jia XY, Briese T, Jordan I, Rambaut A, Chi HC, Mackenzie JS, et al. Genetic analysis of West Nile New York 1999 encephalitis virus. *Lancet* 1999;354:1971-2.
6. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 1999;286: 2333-7.
7. Centers For Disease Control And Prevention. Update: Surveillance for West Nile virus in overwintering mosquitoes--New York, 2000. *MMWR Morb Mortal Wkly Rep* 2000;49:178-9.
8. Nasci R, Savage HM, White DJ, Miller JR, Cropp CB, Godsey MS, et al. West Nile virus in overwintering *Culex* mosquitoes, New York City, 2000. *Emerg Infect Dis* 2001; 7:742-4.
9. De Madrid AT, Porterfield JS. The flaviviruses (group B arboviruses): a cross-neutralization study. *J Gen Virol* 1974;23:91-6.
10. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, et al. Antigenic relationships between flaviviruses as determined by cross- neutralization tests with polyclonal antisera. *J Gen Virol* 1989;70:37-43.
11. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ, 2002. West Nile virus. *Lancet Infect Dis* 2002;2:519-29.
12. Hubalek Z, Halouzka J. West Nile fever--a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis* 1999;5: 643-50.
13. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI, for the Investigative Team. West Nile encephalitis epidemic in southeastern Romania. *Lancet* 1998;352:767-71.
14. Platanov AE, Shipulin GA, Shipulina OY, Tyutyunnik EN, Frolochkina TI, Lanciotti RS, et al. Outbreak of West Nile virus infection, Volgograd region, Russia, 1999. *Emerg Infect Dis* 2001;7:128-32.
15. Centers For Disease Control And Prevention. Guidelines for surveillance, prevention, and control of West Nile virus infection--United States. *MMWR Morb Mortal Wkly Rep* 2000;49: 25-8.

16. Gubler DJ, Campbell GL, Petersen L, Komar N, Nasci RS, Roehrig JT. West Nile virus in the United States: Guidelines for detection, prevention and control. *Viral Immunol* 2000;13:469-75.
17. Centers For Disease Control And Prevention. Preventing emerging infectious diseases: a strategy for the 21st century. Atlanta, GA: U.S. Department of Health and Human Services, 1998.
18. Centers For Disease Control And Prevention. Guidelines for arbovirus surveillance in the United States. Fort Collins, CO: U.S. Department of Health and Human Services, 1993. Available from: URL: <http://www.cdc.gov/ncidod/dvbid/arbor/arboquid.htm>.
19. Eidson, M, Komar N, Sorhage F, Nelson R, Talbot T, Mostashari F, et al. Crow deaths as a sentinel surveillance system for West Nile virus in the Northeastern United States, 1999. *Emerg Infect Dis* 2001;7:615-20.
20. Guptill SC, Julian KG, Campbell GL, Price SD, Marfin AA. Early-season avian deaths from West Nile virus as warnings of human infection. *Emerg Infect Dis* 2003;9:483-4.
21. Julian KG, Eidson M, Kipp AM, Weiss E, Petersen LR, Miller JR, et al. Early season crow mortality as a sentinel for West Nile virus disease in humans, northeastern United States. *Vector Borne Zoonotic Dis* 2002;2:145-55.
22. Mostashari F, Kulldorff M, Hartman JJ, Miller JR, Kulasekera V. Dead bird clusters as an early warning system for West Nile virus activity. *Emerg Infect Dis* 2003;641-6.
23. Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, Manduca RM, et al. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet Pathol* 2000;37: 208-24.
24. Panella NA, Kerst AJ, Lanciotti RS, Bryant P, Wolf B, Komar N. Comparative West Nile virus detection in organs of naturally infected American crows (*Corvus brachyrhynchos*). *Emerg Infect Dis* 2001;7:754-5.
25. Kramer LD, Bernard KA. West Nile virus infection in birds and mammals. *Ann N Y Acad Sci* 2001;951:84-93.
26. Komar N, Lanciotti R, Bowen R, Langevin S, Bunning M. Detection of West Nile virus in oral and cloacal swabs collected from bird carcasses. *Emerg Inf Dis* 2002;8:741-2.
27. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. Rapid detection of West Nile virus from human clinical specimens, field- collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 2000;38:4066-71.
28. Hunt AR, Hall RA, Kerst AJ, Nasci RS, Savage HM, Panella NA, et al. Detection of West Nile virus antigen in mosquitoes and avian tissues by a monoclonal antibody-based capture enzyme immunoassay. *J Clin Microbiol* 2002;40: 2023-30.
29. Ryan J, Dave K, Emmerich E, Fernandez B, Turell M, Johnson J, et al. Wicking assays for

the rapid detection of West Nile and St. Louis encephalitis viral antigens in mosquitoes (Diptera: Culicidae). *J Med Entomol* 2003;40 :95-9.

30. Julian KG, Eidson M, Kipp AM, Weiss A, Petersen LR, Miller JR, et al. Early season crow mortality as a sentinel for West Nile virus disease in humans, Northeastern United States. *Vector Borne and Zoonotic Dis* 2002;2:145-55.
31. Eidson M, Kramer L, Stone W, Hagiwara Y, Schmidt K, New York State West Nile Virus Avian Surveillance Team. Dead bird surveillance as an early warning system for West Nile virus. *Emerg Infect Dis* 2001;7 631–35.
32. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Inf Dis* 2003;9:311-22.
33. Nasci RS, Komar N, Marfin AA, Ludwig GV, Kramer LD, Daniels TJ, et al. Detection of West Nile virus-infected mosquitoes and seropositive juvenile birds in the vicinity of virus-positive dead birds. *Am J Trop Med Hyg* 2002;67:492-6.
34. Guptil SC, Julian KG, Campbell, GL, Price SD, Marfin AA. Early-season avian deaths from West Nile virus as warnings of human infection. *Emerg Infect Dis* 2003;9:483-4.
35. Mostashari F, Kuldorff M, Hartman JJ, Miller JR, Kulasekera V. Dead bird clusters as an early warning system for west nile virus activity. *Emerg Infect Dis* 2003;9:641-6.
36. Komar N. West Nile virus surveillance using sentinel birds. *Annals N Y Acad Sci* 2001;951: 58-73.
37. Beaty BJ, Calisher CH, Shope RE. Arboviruses. In: Lennette EH, Lennette DA, Lennette ET, editors. *Diagnostic procedures for viral, rickettsial, and chlamydial infections*. 7th ed. Washington: American Public Health Association; 1995; p. 189–212.
38. Holden P, Muth D, Shriner RB. Arbovirus hemagglutinin-inhibition in avian sera: inactivation with protamine sulfate. *Am J Epidemiol* 1966;84:67-73.
39. Johnson AJ, Langevin S, Wolff KL, Komar N. Detection of anti-West Nile virus immunoglobulin M in chicken serum by an enzyme-linked immunosorbent assay. *J Clin Microbiol* 2003;41:2002-7.
40. Langevin, SA, Bunning M, Davis B, Komar N. Experimental infection of chickens as candidate sentinels for West Nile virus. *Emerg Infect Dis* 2001;7:726-9.
41. Komar N, Panella NA, Burns, JE, Dusza SW, Mascarenhas TM, Talbot TO. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. *Emerg Infec Dis* 2001;7:621-5.
42. Komar N, Burns J, Dean C, Panella NA, Dusza S, Cherry B. Serological evidence for West Nile virus infection in birds in Staten Island, New York after an outbreak in 2000. *Vector Borne Zoonotic Dis* 2001;1:191-6.
43. Bernard KA, Maffei JG, Jones SA, Kauffman EB, Ebel G, Dupuis AP 2nd, et al and the NY

State West Nile Virus Surveillance Team. West Nile virus infection in birds and mosquitoes, New York State, 2000. *Emerg Infect Dis* 2001;7:679-85.

44. Ebel GD, Dupuis AP 2nd, Nicholas D, Young D, Maffei J, Kramer LD. Detection by enzyme-linked immunosorbent assay of antibodies to West Nile virus in birds. *Emerg Infect Dis* 2002;8:979-82.
45. Blitvich BJ, Marlenee NL, Hall RA, Calisher CH, Bowen RA, Roehrig JT, et al. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. *J Clin Microbiol* 2003;41:1041-7.
46. Roehrig JT, Nash D, Maldin B, Labowitz A, Martin DA, Lanciotti RS, et al. Persistence of virus-reactive serum immunoglobulin m antibody in confirmed west nile virus encephalitis cases. *Emerg Infect Dis* 2003;9:376-9.
47. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet* 2001;358:261-4.
48. Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Atlanta, GA: U.S. Department of Health and Human Services, 2000.
49. Monath TP, Nystrom RR, Bailey RE, Calisher CH, Muth DJ. Immunoglobulin M antibody capture enzyme-linked immunosorbent assay for diagnosis of St. Louis encephalitis. *J Clin Microbiol* 1984;20:784-90.
50. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;38:1823-6.
51. Johnson AJ, Martin DA, Karabatsos N, Roehrig JT. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. *J Clin Microbiol* 2000;38:1827-31.
52. Shieh WJ, Guarner J, Layton M, Fine A, Miller J, Nash D, et al. The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999. *Emerg Infect Dis* 2000;6:370-2.
53. Tsai TF, Bolin RA, Montoya M, Bailey RE, Francy DB, Jozan M, et al. Detection of St. Louis encephalitis virus antigen in mosquitoes by capture enzyme immunoassay. *J Clin Microbiol* 1987;25:370-6.
54. Tsai TF, Happ CM, Bolin RA, Montoya M, Campos E, Francy DB, et al. Stability of St. Louis encephalitis viral antigen detected by enzyme immunoassay in infected mosquitoes. *J Clin Microbiol* 1988;26:2620-5.
55. Briese T, Glass WG, Lipkin WI. Detection of West Nile virus sequences in cerebrospinal fluid. *Lancet* 2000;355:1614-5.

56. Shi PY, Kauffman EB, Ren P, Felton A, Tai JH, Dupuis AP 2nd, et al. High-throughput detection of West Nile virus RNA. *J Clin Microbiol* 2001;39:1264-71.
57. Lanciotti RS, Kerst AJ. Nucleic Acid Sequence-Based Amplification Assays for Rapid Detection of West Nile and St. Louis Encephalitis Viruses. *J Clin Microbiol* 2001;39:4506-13.
58. Rose RI. Pesticides and public health: Integrated methods of mosquito management. *Emerg Infect Dis* 2001;7:17-23.
59. American Mosquito Control Association and Environmental Protection Agency. Environmental Protection Agency's Pesticide Environmental Stewardship Program Partnership Strategy for the American Mosquito Control Association, 1997. Available from: URL: [www.mosquito.org/PESPAMCA.htm](http://www.mosquito.org/PESPAMCA.htm).
60. Florida Coordinating Committee Mosquito Control. Florida mosquito control: The state mission as defined by mosquito controllers, regulators, and environmental managers. Gainesville, FL: University of Florida, 1998. Available from: URL: [www.ifas.ufl.edu/~veroweb/whitep/whitep.htm](http://www.ifas.ufl.edu/~veroweb/whitep/whitep.htm).
61. New Jersey Mosquito Control Association. New Jersey Mosquito Control Association partnership strategy document: Environmental Protection Agency Pesticide Environmental Stewardship Program. New Brunswick, NJ: Cook College, 1997. Available from: URL: [www.rci.rutgers.edu/~insects/psd.html](http://www.rci.rutgers.edu/~insects/psd.html).
62. Service MW. Mosquito ecology: Field sampling Methods. New York, NY: John Wiley and Sons; 1976.
63. Newhouse VR, Chamberlain RW, Johnston JF, Sudia WD. Use of dry ice to increase mosquito catches of the CDC miniature light trap. *Mosquito News* 1966;26:30-5.
64. Reiter PA. A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. *Mosquito News* 1983;43:496-8.
65. Reiter P, Jakob WL, Francy DB, Mullenix JB. Evaluation of the CDC gravid trap for the surveillance of St. Louis encephalitis vectors in Memphis, Tennessee. *J Am Mosq Control Assoc* 1986;2:209-11.
66. Andis MD, Sackett SR, Carroll MK, Bordes ES. Strategies for the emergency control of arboviral epidemics in New Orleans. *J Am Mosq Control Assoc* 1987;3:125-30.
67. Leiser LB, Beier JC, Craig GB. The efficacy of malathion ULV spraying for urban *Culex* control in South Bend, Indiana. *Mosquito News* 1982;42:617-8.
68. Mitchell CJ, Hayes RO, Holden P, Hill HR, Hughes TB Jr. Effects of ultra-low volume applications of malathion in Hale County, Texas. I. Western encephalitis virus activity in treated and untreated towns. *J Med Entomol* 1969;6:155-62.
69. Mitchell CJ, Kilpatrick JW, Hayes RO, Curry HW. Effects of ultra-low volume applications of malathion in Hale County, Texas. II. Mosquito populations in treated and untreated areas. *J Med Entomol* 1970;7:85-91.

70. Mount GA, Biery TL, Haile DG. A review of ultra-low aerial sprays of insecticide for mosquito control. *J Am Mosq Control Assoc* 1966;12:601-18.
71. Reisen WK, Milby MM, Reeves WC, Eberle MW, Meyer RP, Schaefer CH, et al. Aerial adulticiding for the suppression of *Culex tarsalis* in Kern County, California, using low volume propoxur: 2. Impact on natural populations in foothill and valley habitats. *J Am Mosq Control Assoc* 1985;1:154-63.
72. Brogdon WG, McAllister JC. Insecticide resistance and vector control. *Emerg Infect Dis* 1998;4:605-13.
73. Lum MR, Tinker TL. A primer on health risk communication principles and practices. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, 1994. Available from: URL: <http://www.astdr.cdc.gov/HEC/primer.html>.
74. Hopkins CC, Hollinger FB, Johnson RF, Dewlett HJ, Newhouse VF, Chamberlain RW. The epidemiology of St. Louis encephalitis in Dallas, Texas, 1966. *Am J Epidemiol* 1975;102:1-15.
75. Monath TP. Epidemiology. In: Monath TP, editor. *St. Louis encephalitis*. Washington: American Public Health Association; 1980. p. 239-312.
76. Covello VT, Allen FW. *Seven cardinal rules of risk communication*. Washington: U.S. Environmental Protection Agency, 1988.
77. Lum MR, Tinker TL. A primer on health risk communication principles and practices. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, 1994. Available from: URL: [www.astdr.cdc.gov/HEC/primer.htm](http://www.astdr.cdc.gov/HEC/primer.htm).