Centers for Disease Control and Prevention Epidemic Epizootic West Nile Virus in the United States: Revised Guidelines for Surveillance, Prevention, and Control

From a Workshop
Held in Charlotte, North Carolina,
January 31 - February 4, 2001

CDC
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U.S. Department of Health and Human Services
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Centers for Disease Control and Prevention
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Division of Vector-Borne Infectious Diseases
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Executive Summary

In late summer 1999, the first domestically acquired human cases of West Nile (WN) encephalitis were documented in the U.S. The discovery of virus-infected, overwintering mosquitoes during the winter of 1999-2000 predicted renewed virus activity for the following spring and launched early season vector-control and disease surveillance in New York City and the surrounding areas. These surveillance efforts were focused on identifying and documenting WN virus infections in birds, mosquitoes and equines as sentinel animals that could predict the occurrence of human disease. By the end of the 2000 transmission season, WN virus activity had been identified in a 12 state area from Vermont and New Hampshire in the north to North Carolina in the south. In 2000 there were 21 humans cases, 63 horses, 4,304 birds (78 species including 1999 data), and 480 mosquito pools (14 species) reported with WN virus. This annual human case incidence now ranks WN virus second only to LaCrosse encephalitis virus as the leading cause of reported human arboviral encephalitis in the U.S.

To assess the implications of the WN virus introduction into the U.S. and to develop a comprehensive national response plan, the Centers for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture (USDA) co-sponsored a meeting of arbovirologists, epidemiologists, laboratorians, vector-control specialists, wildlife biologists, and state and local health and agriculture officials in Fort Collins, Colorado, on November 8-9, 1999. As an outgrowth of this meeting, recommendations for surveillance, prevention and control of WN virus in the U.S. were developed, published and used in 2000 by federal, state and local public health officials. A second national meeting, co-sponsored by CDC, the Association of Public Health Laboratories and other federal and state organizations was held in Charlotte, North Carolina, on January 31-February 4, 2001, to review year 2000 WN virus activity, and evaluate the outcomes of recommended surveillance, prevention and control activities. Sessions were organized to review each major guideline topic. Each session was comprised of summary talks followed by a panel discussion of experts from around the U.S. Each topic-oriented discussion group met to propose modifications to their guideline topic. A copy of the 2001 meeting agenda and participant list are attached to this report as Appendix A. Based on the results of this second meeting, modified Guidelines were formulated. This document is available on the CDC Internet Web page at:
http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm.

Surveillance

Enhanced surveillance is a high priority for those states that are affected or that are at higher risk for being affected by WN virus because of bird migration patterns and virus spread. These include states from Maine, New Hampshire and Vermont to Texas along the Atlantic and Gulf coasts, states immediately adjacent to states with current WN virus activity, Canada and countries in the Caribbean and Central and South America. Depending on the geographic location of the state, active surveillance should be implemented in the spring and continued until the late fall (for states where mosquito activity will cease because of cold weather) or through the winter months (for southern states where mosquito activity may be continuous throughout the year). In all states that face potential WN virus activity, the following surveillance activities should be emphasized:

1. Active bird surveillance. Arbovirus activity should be monitored in wild birds, sentinel birds, or both. Surveillance for dead crows and other members of the family Corvidae, in particular, is a sensitive means to detect the presence of WN virus in a geographic area. For some areas, however, crows might not be the first birds identified with WN virus infection.

2. Active mosquito surveillance. Surveillance of mosquito populations should be initiated to detect WN and other arbovirus activity, to help identify potential mosquito vectors in a particular area and to monitor population densities of those vectors. In 1999, WN virus infections were found mainly in bird feeding mosquitoes. In 2000, WN virus-infected mammal feeding mosquitoes were also identified.

3. Enhanced passive veterinary surveillance. As a backup system to detect the presence of WN virus and to monitor the extent of its transmission outside the bird-mosquito cycle, enhanced passive surveillance (passive surveillance enhanced by general alerts to veterinarians) for neurologic disease in horses and other animals should be implemented. In 2000, human infections temporally preceded horse infections; the reason for this is not known.

4. Enhanced passive human surveillance. As a backup system to detect the presence of WN virus activity, enhanced passive surveillance (passive surveillance enhanced by general alerts to health-care providers) for human cases of viral encephalitis and, if resources permit, aseptic meningitis should be implemented.

Laboratory Diagnosis

Unequivocal diagnosis of WN virus or other arbovirus infection requires specialized laboratory diagnostic tests. Success of surveillance activities is dependent on the availability of laboratories that can provide diagnostic support. The following minimal laboratory support is critical. CDC and USDA have and will continue to provide reagents and training as needed.

1. Serology. The immunoglobulin (Ig) M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) should be available in all state public health and veterinary laboratories to provide first-line testing for human and animal serum and cerebrospinal fluid specimens. In addition, selected state health, veterinary, and reference laboratories should have the capability to do neutralization tests to identify specific flavivirus antibody.

2. Virus isolation and detection. Selected state public health and reference laboratories should have virus isolation and identification capabilities. Well defined real-time polymerase chain reaction (PCR) assays have been developed, published and implemented. Selected laboratories should have PCR capability to detect viral RNA. For those laboratories that cannot make the financial commitment to PCR, others assays like direct RNA detection and antigen-capture ELISAs to detect WN and St. Louis encephalitis viruses in mosquito pools and avian tissues are available. All laboratory protocols that require handling live virus, or tissues possibly containing live virus, should be conducted under biosafety level containment as recommended by the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* manual. Finally, selected state public health

and reference laboratories should have the capability to do immunohistochemistry to detect WN virus in autopsy tissues.

Prevention and Control

Currently, the most effective way to prevent transmission of WN virus and other arboviruses to humans and other animals, or to control an epidemic once transmission has begun, is to reduce human exposure via mosquito control. To prevent human and domestic animal disease, state and local health departments must have adequate mosquito control capabilities. A response algorithm based upon WN virus activity is given in Table 1 of the Guidelines.

1. Mosquito abatement districts. The most effective and economical way to control mosquitoes is by larval source reduction. Experience suggests that this is best done through locally funded abatement programs that monitor mosquito populations and initiate control before disease transmission to humans and domestic animals occurs. These programs can also be used as the first-line emergency response for mosquito control if and when virus activity is detected in an area or human disease is reported. Control of adult mosquito populations by aerial application of insecticides is usually reserved as a last resort.

2. Public outreach. A critical component of any prevention and control program for vector-borne diseases is public education about these diseases, how they are transmitted and how to prevent or reduce risk of exposure. Public education should utilize behavioral science and social marketing methods to effectively communicate information to target populations.

Public Health Infrastructure

Effective surveillance, prevention and control of vector-borne diseases, including disease caused by WN virus, may require a re-evaluation of resource priorities in local and state health departments. Currently, only a few states and even fewer local health departments have trained personnel or the resources to adequately address vector-borne diseases. Every state health department should have, at a minimum, a functional arbovirus surveillance and response capability, including entomology and veterinary health capacity and an adequately equipped laboratory with trained staff. Ultimately, the annual risk of arbovirus activity will determine the extent of a state's activities to deal with arbovirus diseases.

Interjurisdictional Data Sharing

WN virus is a zoonosis that affects a number of animal species, including humans. Effective surveillance and response require close coordination and data exchange between many agencies, including federal, state and local public health, vector control, agriculture and wildlife departments. Information and data exchange can be facilitated through a system of secure electronic communication, e.g., list servers and web sites, that can be accessed by authorized users. To assist guideline implementation in 2000, CDC developed an electronic-based

surveillance and reporting system (ArboNet) to track WN virus activity in humans, horses, other mammals, birds and mosquitoes. The details of ArboNet are given in Appendix B of the guidelines.

Research Priorities

Understanding how and why the WN virus epidemic/epizootic occurred, the public health and animal health implications of this introduction to the Western Hemisphere, and development of effective prevention strategies will require considerable research. Some of the high priority research topics include:

- Current and future geographic distribution
- Bird migration as a mechanism of virus dispersal
- Vector relationships and range
- Vertebrate host relationships and range
- Virus persistence mechanisms
- Mosquito biology and behavior
- Mosquito control methodologies
- Mosquito surveillance methodologies
- Development and evaluation of prevention strategies
- Improved laboratory diagnostic tests
- Clinical spectrum of disease and long-term prognosis in humans
- Risk factor studies in enzootic areas
- Viral pathogenesis
- Genetic relationships and molecular basis of virulence
- WN virus vaccine development for animals and humans
- Antiviral therapy for WN virus
- Economic analysis of the epidemic
- Impact on wildlife
- Evaluation of pesticide effects on humans
- Methods of introduction of WN virus into the U.S.

Introduction

In late summer 1999, the first domestically acquired human cases of West Nile (WN) encephalitis were documented in the U.S.[1-6] The discovery of virus-infected, overwintering mosquitoes during the winter of 1999-2000 predicted renewed virus activity for the following spring and launched early season vector-control and disease surveillance in New York City (NYC) and the surrounding areas.[7, 8] These surveillance efforts were focused on identifying and documenting WN virus infections in birds, mosquitoes and equines as sentinel animals that could predict the occurrence of human disease. By the end of the 2000 mosquito-borne pathogen transmission season, WN virus activity had been identified in a 12 state area from Vermont and New Hampshire in the north to North Carolina in the south. In 2000 there were 21 humans, 63 horses, 4,304 birds (78 species including 1999 data), and 480 mosquito pools (14 species) reported with WN virus.[9-12] This annual human case incidence now ranks WN virus second only to LaCrosse encephalitis virus as the leading cause of reported human arboviral encephalitis in the U.S.

West Nile virus is a member of the family Flaviviridae (genus Flavivirus). Serologically it is a member of the Japanese encephalitis virus complex that includes St. Louis encephalitis (SLE), Japanese encephalitis, Kunjin, and Murray Valley encephalitis viruses, as well as others.[13, 14] WN virus was first isolated in the West Nile province of Uganda in 1937.[15, 16] The first recorded epidemics occurred in Israel during 1951-1954, and in 1957. The largest recorded epidemic caused by WN virus occurred in South Africa in 1974. A large human outbreak of WN encephalitis occurred in Israel in 2000. European epidemics of WN encephalitis have occurred in southern France in 1962, in southeastern Romania in 1996, and in south-central Russia in 1999.[17, 18] European equine outbreaks also have occurred in Italy in 1998 and in France in 2000.

Although it is still not known when or how WN virus 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. WN virus can infect a wide range of vertebrates; in humans it usually produces either asymptomatic infection or mild febrile disease, sometimes accompanied by rash, but it can cause severe and fatal infection in a small percentage of patients. In 1999 in New York, approximately 40% of laboratory-positive humans with encephalitis or meningitis had severe muscle weakness; 10% developed flaccid paralysis with electromyographic findings consistent with axonal neuropathy. The human case-fatality rate in the U.S. has been about 11%.

Unlike WN virus within its historical geographic range, or SLE virus in the Western Hemisphere, mortality in a wide variety of bird species has been a hallmark of WN virus in the U.S. The reasons for this are not known; however, public health officials were able to use bird mortality (particularly birds from the family Corvidae) to effectively track WN virus expansion in 2000. Early season field studies determined that areas with bird mortality due to WN virus infection were experiencing ongoing enzootic transmission. However, most birds survive WN virus infection as indicated by the high seroprevalence in numerous species of resident birds within the regions of greatest virus transmission. It is still not known to what degree migrating birds contribute to natural transmission cycles and dispersal of both viruses.

In 1999 WN virus was transmitted principally by *Culex* species mosquitoes, the usual vectors of SLE virus. In 2000, there was a total of 14 WN virus-infected mosquito species (including *Culex* sp.) identified, although 89% of positive mosquito pools were *Culex*. As opposed to *Culex*, many of these other species are daytime feeders and mammal feeders. The effect that this widened spectrum of WN virus-infected mosquito species will have on WN virus ecology in the U.S. is not known. It must be remembered, however, that WN virus-infection does not always implicate a mosquito species as a competent vector of WN virus.

To assess the implications of the WN virus introduction into the U.S. and to develop a comprehensive national response plan, the Centers for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture co-sponsored a meeting of arbovirologists, epidemiologists, laboratorians, vector-control specialists, wildlife biologists, and state and local health and agriculture officials in Fort Collins, Colorado, on November 8-9, 1999. The recommendations of these experts were used to prepare the 2000 Guidelines for Surveillance, Prevention and Control for Epidemic/Enzootic West Nile Virus in the U.S.[19, 20] This document is available on the CDC Internet Web page 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 WN virus activity in humans, horses, other mammals, birds and mosquitoes.

A second national meeting, co-sponsored by CDC, the Association of Public Health Laboratories (APHL) and other federal and state organizations was held in Charlotte, North Carolina, on January 31-February 4, 2001, to review year 2000 WN virus activity, assess surveillance, prevention and control activities, and formulate modified guidelines. Sessions were organized to review each major guideline topic. Each session was comprised of summary talks followed by a panel discussion of experts from around the U.S. Each topic-oriented discussion group met to propose modifications to their guideline topic. A copy of the 2001 meeting agenda and participant list are attached to this report as Appendix A.

Workshop participants agreed that although the 2000 efforts generally were successful, additional improvements in the public health infrastructure to control vector-borne diseases are needed at the local, state, and national level. Today's rapid transport of people, animals, and commodities increase the likelihood that other introductions of exotic pathogens will occur. There was general agreement that CDC should continue to move as quickly as possible to fully implement the plan entitled "Preventing Emerging Infectious Diseases, a Plan for the 21st Century."[21]

I. SURVEILLANCE

A universally applicable arbovirus surveillance system does not exist. In any given jurisdiction, surveillance systems should be tailored according to 1) the probability of arbovirus activity, and 2) available resources. In jurisdictions without pre-existing vector-borne disease programs, newly developed avian-based and/or mosquito-based arbovirus surveillance systems will be required. In some, resurrection of previously abandoned systems is necessary. In others, modification and/or strengthening of existing arbovirus surveillance systems, *e.g.*, for detection of eastern equine encephalitis (EEE), western equine encephalitis (WEE), and/or SLE viruses, 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. States already affected by WN virus, and contiguous states, should initiate surveillance after mosquitoes become active in spring; some states should consider surveillance for infected overwintering mosquitoes. States in the southern part of this area (*e.g.,* Virginia, North Carolina) will find active mosquitoes earlier in spring than those in the northern region. Other states should be on alert for WN virus activity during the period that arboviruses are typically active in their area.

Appropriate and timely response to surveillance data is the key to preventing human and animal disease associated with WN and other arboviruses. That response must be effective mosquito control without delay, if increasing levels of virus activity are detected in the bird or mosquito surveillance systems (see Section III.N). A basic reference on arbovirus surveillance is: CDC Guidelines for Arbovirus Surveillance Programs in the United States.[22] This document can be obtained from the Division of Vector-Borne Infectious Diseases in Fort Collins, Colorado, and is also available on the CDC home page at: www.cdc.gov/ncidod/dvbid/arbor/arboguid.htm.

A. Ecologic Surveillance

Detection of WN virus in bird and mosquito populations is a useful indicator to predict and prevent human and domestic animal infections. Surveillance to detect WN virus should focus on the avian and mosquito components of the enzootic transmission cycle. Non-human mammals may also serve as effective sentinels because their high level of exposure to mosquitoes makes them more likely to be infected than people. Descriptions of the avian, mosquito and non-human mammals surveillance strategies follow:

1. Avian

    a. Avian morbidity/mortality

        Avian morbidity/mortality surveillance appears to be the most sensitive early detection system for WN virus, and should be a component of every state's arbovirus surveillance program. Its utility for monitoring ongoing transmission in a standardized fashion currently is being investigated, but should include at least two basic elements: 1) the timely reporting and analysis of dead bird sightings and 2) submission of selected individual birds for WN virus testing.

        GOAL

        Utilize bird mortality associated with WN virus infection as a means of detecting WN virus activity in a location.

        (1) Protocols and specimens

The level of effort in this surveillance activity will depend on the risk assessment for 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. Birds in good condition (without obvious decomposition, scavenged or infested with maggots) 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.[23] The number of bird specimens tested will be dependent upon resources and whether WN virus-infected birds have been found in the area; triage of specimens may be necessary on the basis of sensitive species and geographic location.

A single organ specimen from each bird is sufficient to detect WN virus or viral RNA. Kidney, brain or heart is preferable. Testing involves isolation of infectious virus, or specific RNA detection by RT-PCR, and will generally identify an infection within one - two weeks after transmission.[24] For confirmation of initial positive findings in a new geographic area, additional testing is encouraged.

(2) Recent experience:

Analysis of avian morbidity and mortality data in 2000 indicated:

(a) American crow was the most sensitive species for avian morbidity/mortality surveillance. However, some areas, particularly those distant from NYC, did not have positive American crows, but only positive birds of other species.

(b) Almost all of the positive birds were found singly and not as part of a mass die-off in a single time and place.

(c) Approximately a third of the positive birds had signs of trauma on necropsy.

(d) Many positive birds did not have pathology indicative of WN infection on necropsy. No lesions are pathognomonic for WN virus infection.

(e) Positive dead birds usually provided the earliest indication of viral activity in an area.

(f) Detection of positive dead birds always preceded reporting of human cases (although knowledge of the test result did not necessarily predate onset of the human case).

(g) Those counties with human cases tended to have high dead bird surveillance indices, both WN virus positive and sightings.

(h) Experimental evidence of direct transmission among crows (USGS-National Wildlife Health Center, unpublished data) has been reported which may alter interpretation of WN virus surveillance findings if this phenomenon occurs in nature.

(3) Advantages of avian morbidity/mortality surveillance:

(a) Certain species of birds, in particular corvids (*e.g.,* crows and jays) appear to experience high clinical attack rates.

(b) The size and coloration of certain dead birds make them conspicuous (*e.g.*, crows).

(c) RT-PCR can be used to rapidly detect WN viral RNA in tissues, even if grossly decomposed.

(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) Provides temporally and spatially sensitive detection of WN virus activity.

(f) Multiple findings of WN virus in dead birds likely represents local transmission.

(g) Can be used for early detection and possibly also for ongoing monitoring of WN virus transmission.

(h) May be used to estimate risk of human infection.

(4) Disadvantages of avian morbidity/mortality surveillance:

(a) Dead bird surveillance data among 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.

(c) Collection, handling, shipping, and processing of birds or their clinical specimens are 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.

(f) Success is influenced by public participation, which is highly variable, and depends on the amount of public outreach programs, 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.

b. Live birds

Live bird surveillance has been used traditionally both to detect and monitor arbovirus transmission (*e.g.*, for SLE, EEE and WEE viruses). Two surveillance approaches are 1) captive sentinel surveillance, typically using chickens, but other species have been used as well, and 2) free-ranging bird surveillance. Both depend on serological testing, which generally requires at least 3 weeks to detect and confirm an infection. Neither of these surveillance systems have been adequately evaluated for use in North America. Successful applications of these systems 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 WN surveillance.

GOAL

Utilize seroprevalence in captive or free-ranging bird species as qualitative indicators of local WN virus activity.

(1) Captive sentinel surveillance

Although an ideal captive avian sentinel for WN virus -- or any other arbovirus – may not exist, such a species would meet the following criteria: 1) universal susceptibility to infection, 2) 100% survival from infection as well as universal development of easily detectable antibodies, 3) poses no risk of infection to handlers, and 4) never develops viremia sufficient to infect vector mosquitoes.[22] Captive sentinels have been effective means of monitoring 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 congregation sites), and presented appropriately to allow feeding by enzootic WN virus vectors. Alternatively, pre-existing captive birds (*e.g.,* domestic poultry, pigeons and zoologic collections) may be used as sentinels.

(a) Protocols and specimens

Whole blood can be collected in microtainers and centrifuged for serum. Serum is screened by either hemagglutination inhibition (HI), enzyme-linked immunosorbent assay (ELISA) or plaque-reduction neutralization test (PRNT). It is important to note that extraction of avian serum samples for use in the HI test follows procedures different from human serum samples.[25] Positive tests must be confirmed by neutralization to

rule out false positives and cross-reaction due to infection with related flaviviruses (*e.g.,* SLE). All jurisdictions will be required to have institutional animal care and use protocols.

(b) Recent Experiences:

1) Chickens were used as sentinels in 2000 in selected counties in NY, NYC, NJ, PA, MD and DE. Small numbers of seroconversions were detected late in the season in NJ and NY. As used, chickens were ineffective sentinels in 2000.

2) MAC-ELISA testing of experimentally infected chickens points to the need for weekly sampling of sentinels.

3) Experimental studies have shown that chickens, pigeons and pheasants (CDC, unpublished data) are candidate sentinels due to their susceptibility to infection, low mortality and incompetence as reservoirs. However, small amounts of WN virus were detected in cloacal swabs from infected chickens and pigeons.

4) Field studies of avian seroprevalence in Queens in 1999 indicated that captive chickens frequently were infected. In Staten Island in 2000, captive pigeons frequently were infected (CDC, NYCDOH unpublished data).

5) Some mortality in chickens was attributed to WN virus at various locations in New York State.

(c) Advantages of sentinel captive bird surveillance:

1) There is a long history (> 6 decades) of successful use in flavivirus surveillance (chickens).

2) Birds are readily fed upon by WN virus vector mosquitoes.

3) Captive birds can be serially bled, and the geographic location of infection is not in question.

4) The system is flexible and therefore can be expanded and contracted as appropriate.

5) Flocks can be bled, maintained and specimens submitted for testing by mosquito-abatement districts.

6) Collection of specimens is inexpensive compared to free-ranging bird surveillance.

(d) Disadvantages of captive sentinel surveillance:

1) More research is needed to validate the usefulness of sentinel captive birds for monitoring WN transmission.

2) Sentinel flocks detect only focal transmission, requiring that multiple flocks be positioned in representative geographic areas. This is particularly true when vector mosquitoes have short flight ranges (*e.g., Culex pipiens*).

3) Flocks are subject to vandalism and theft.

4) Flocks must be protected from predators.

5) Set-up and flock maintenance are expensive (*i.e.*, birds, cages, feed, transportation). Training is required for proper maintenance and sampling.

6) Pre-existing flocks may already be exposed due to previous local WN virus transmission.

(2) Free-ranging bird surveillance

The use of free-ranging live birds provides 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 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 like the plaque-reduction neutralization test (PRNT) will need to be used to detect WN virus-specific antibody. Birds aged <1 year old with antibodies may be presumed infected recently (within the current transmission season). Weak seropositivity in very young birds (<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. Some

jurisdictions may require institutional animal care and use protocols for the initiation of wild bird sampling programs. State and federal permits are required.

(b) Recent experience:

1) In urban epizootic transmission foci in NYC, several common species developed high seroprevalence, making them strong candidate sentinels (*e.g.,* house sparrows, cardinals, catbirds, mourning doves, rock doves), although other species may be important in other locations.

2) High seroprevalence of important reservoir hosts (*e.g.*, house sparrows) in northeastern Queens in 1999 preceded low transmission activity to humans in the same neighborhoods in 2000.

(c) Advantages

1) Long history of successful use in flavivirus surveillance.

2) Local movement of resident wild birds may allow contact with enzootic transmission foci, thus increasing sensitivity.

3) Set-up or maintenance costs may be minimal.

4) Highly flexible sampling capability.

5) Permits evaluation of herd immunity among important amplifier hosts.

6) Owner confidentiality might be less of an issue.

(d) Disadvantages

1) More research is needed to validate the usefulness of free-ranging sentinels for monitoring WN transmission.

2) Interpretation is complex.

3) Handling and venipuncture of reservoir species increases the risk of exposure to pathogens in feces and by accidental needle stick.

4) Movement of free-ranging wild birds makes it impossible to know where the infection was acquired.

5) Most birds are protected by federal law and their possession requires state and federal permits. Banding permits require complex data reporting.

6) Training is required for live-trapping, blood-sampling, handling and accurate determination of species and age of wild birds.

7) It is generally not feasible to serially bleed individual free-ranging birds because of low recapture rates (although banding can be useful).

2. Equines

Surveillance for WN virus disease in equines should be conducted, because they are potential sentinels of WN virus epizootic activity, and equine health is an important economic issue. Although equines did not appear to be good sentinels of increased human risk for WN virus infection in 1999-2000, more experience is clearly needed. Veterinarians and veterinary service societies/agencies are essential partners in any surveillance activities involving equines with WN virus disease. Any utility of equines as sentinels of increased human risk may soon be reduced if equine WN virus vaccines become available. A working surveillance case definition of clinical WN virus infection in equines is presented in Appendix C.

GOAL

To 1) assess the public and equine health impact of WN virus disease and monitor national trends, 2) demonstrate the need for public and animal health intervention programs and resources and allocate resources, and 3) identify factors for high-risk population groups or geographic areas to target interventions.

(1) Protocols and Specimens

(a) Serum and CSF for antibody testing.

(b) Necropsy tissues (especially brain and spinal cord) for gross pathology, histopathology, RT-PCR, virus isolation, and immunohistochemistry.

(2) Advantages

(a) Horses are highly conspicuous, numerous, and widely distributed in some areas.

(b) Some are routinely bled and tested for other pathogens.

(c) Ill horses may be the first indication of WN virus activity in rural areas.

(3) Disadvantages

(a) Horses are usually not a good “early warning” sentinel, because like humans they are infected tangentially to the primary cycle (*i.e.*, human cases of EEE may occur simultaneously with or soon after horse cases).

(b) Necropsies are expensive and logistically difficult.

(c) Horses are not present or abundant in many areas of the U.S..

3. Mosquitoes

Mosquito surveillance, along with bird-based surveillance, should be the mainstay of most surveillance programs for arboviruses, including WN virus.

GOAL

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 intervention events, 4) identify larval habitats for targeted control, 5) monitor the effectiveness 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 for species identification and for virus detection.

(2) Larval mosquitoes for species identification and habitat mapping.

b. Recent experience

(1) If mosquito trapping effort is inadequate, WN virus-positive mosquitoes may not be detected prior to the identification of a human WN virus case.

(2) Avian epizootics can occur without having demonstrable human WN virus infection. The epizootics are demonstrated, in part, by detection of WN virus positive mosquito pools containing only species that feed predominantly on birds.

(3) On Staten Island, where numerous human cases occurred, intense epizootic transmission involving both birds and mammals was demonstrated. High infection rates were found in mosquito species that feed on birds, and multiple WN virus positive pools were found in species that feed on mammals.

c. Advantages

(1) May provide the earliest evidence of transmission in an area.

(2) Provides information on potential mosquito vector species.

(3) Provides an estimate of vector species abundance.

(4) Provides information on virus infection rates in different mosquito species.

(5) Provides information on potential risk to humans and animals.

(6) Provides baseline data that can be used to guide emergency control operations.

(7) Allows evaluation of control methods.

d. Disadvantages

(1) 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.

e. Outline of Minimum Entomological Surveillance Program

A mosquito-based surveillance program will vary by geography and availability of financial and personnel resources. To assure long-term continuous operation, a constant source of funding is necessary.

A mosquito surveillance program should be developed using the guidelines presented below in outline format. A network of fixed trap sites is necessary for the development of a database that would allow temporal and spatial evaluation of changes in mosquito population size. It may take one entire transmission season to identify the best fixed sites in an area and to establish the fixed network. A flexible trapping system will maximize the likelihood of obtaining virus isolates, for example, by moving traps to sites near suspect human cases or sites of crow deaths. Flexible trapping is also necessary if mosquito counts are used to evaluate targeted control efforts. It is advantageous for an entomological surveillance program to have the ability to use both sampling strategies.

(1) Obtain basic literature and expertise on mosquito identification, biology and surveillance.

(2) Develop contacts with established regional mosquito surveillance programs, and local and nation mosquito associations.

(3) Surveillance Program

(a) Larval surveillance
    1) Determine species present
    2) Characterize and map larval habitats by season

(b) Adult surveillance
    1) Use both fixed and flexible trap positions if possible
        a) Fixed positions allow for the development of a database that would allow for comparison of population data to previous years and the spatial mapping of changes in mosquito abundance
        b) Flexible sites allow for response to epidemiological and natural events, *e.g.*, a suspect human case, dead crow, or flood event
        c) Use a variety of trapping methods
            i. CDC light traps baited with CO_2
            ii. Gravid traps
            iii. Other methods, ovicups, aspirators etc
        d) Trap distribution will be influenced by several species factors:
            i. Habitat diversity, size and abundance
            ii. Resource availability
            iii. Proximity to human population centers and/or recreational areas
            iv. Flight range of vector species

(c) Virus surveillance
    1) Determine infection rates by species
        a) Make arrangements with lab for testing
        b) Focus initially on *Culex* mosquitoes to provide first indication of WN virus presence
        c) Once virus is detected in *Culex* mosquitoes, pool and test all potential vector species with emphasis on incriminated or suspected species

(4) Establish database and analyze data on regular basis to evaluate disease risk, and to direct and evaluate control efforts. Establish electronic data bases using GIS format to provide a spatial appreciation of anomalies in population measurements.

(5) Share results with cooperating public health agencies and other mosquito control districts.

B. Surveillance for Human Cases

Because the primary public health objective of surveillance systems for encephalitis-causing arboviruses is the prevention of human infections and disease, human case surveillance alone should not be used for the detection of arbovirus activity, except in jurisdictions where 1) arbovirus activity is considered to be of very low likelihood, or 2) resources to support avian-based and/or mosquito-based arbovirus surveillance are unavailable.

GOAL

To 1) assess the local, state and national public health impact of WN virus disease and monitor national trends, 2) demonstrate the need for public health intervention programs and allocate resources and 3) identify risk factors for infection and high-risk population groups or geographic areas to target interventions and guide analytic studies.

1. Recent Experience

   a. In the United States during 1999-2000, the peak human risk for WN viral infection occurred in mid- to late August, and seemed to rapidly decline after the first week in September. The highest measured minimum infection rates in mosquitoes, and the majority of equine cases of WN viral infection, occurred after the apparent decrease in human risk.

   b. In 1999-2000, the large majority of confirmed cases of human illness due to WN virus infection were in persons with encephalitis. Testing of patients with aseptic meningitis or Guillain-Barrè syndrome for evidence of WN viral infection was low-yield.

   c. Most patients with WN encephalitis or meningitis are older adults, generally aged >50 years. In the United States, the median age of hospitalized patients in 1999 was 71 years; in 2000, it was 63 years. Such cases in children are unusual.

   d. In the United States, WN encephalitis has been associated with a Guillain-Barrè-like syndrome with generalized muscle weakness. In 1999, generalized muscle weakness was seen in 34% of WN encephalitis cases; in 2000, it was seen in 16% of such cases.

   e. Using CDC-recommended test methods in public health laboratories, WN virus-specific IgM antibody was detected in acute-phase (i.e., those collected $\leq$8 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 WN encephalitis cases have shown that WN virus-specific IgM antibody can persist in serum for 12 months or longer. Thus, the presence of serum anti-WN viral IgM antibody is not necessarily diagnostic of *acute* WN viral infection. For this reason, especially in areas where WN virus is known to have circulated previously, suspected cases of acute WN encephalitis or meningitis should be confirmed by the demonstration of WN virus-specific IgM antibody in CSF, the development of WN virus-specific IgG antibody in convalescent-phase serum, or both.

g. In the United States, the sensitivity of PCR tests of CSF for the diagnosis of human WN encephalitis cases was only 57% in 1999. 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 virus-specific antibody in CSF and serum, tests which are far more sensitive.

2. Types of Surveillance

a. Clinical Syndromes to Monitor

In general, monitoring of encephalitis cases is the highest priority. Monitoring of milder illnesses such as aseptic meningitis, Guillain-Barré syndrome, and fever with rash illness is resource-dependent and should be of lower priority.

b. Types of Human Surveillance

(1) Enhanced passive surveillance

In the absence of known WN virus activity in an area, enhanced passive surveillance* for hospitalized cases of encephalitis of unknown etiology,** and for patients who test positive for antibodies to either WN or SLE virus in commercial or government laboratories, should be employed. A high index of suspicion for arboviral encephalitis should be encouraged. When in doubt, appropriate clinical specimens should be submitted to CDC or another laboratory capable of reliably diagnosing arboviral infections. It is important that paired acute- and convalescent-phase serum samples be submitted to ensure accurate interpretation of serologic results.

(2) Active surveillance

Active surveillance should be considered in areas with known WN virus activity. In general, one or both of the following approaches should be taken: 1) Identify physicians in appropriate specialties (*e.g.*, infectious diseases, neurology, and intensive care medicine) and hospital infection control personnel and contact them on a regular basis to inquire about

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*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 febrile illnesses associated with a wide range of neurologic manifestations. These 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 be clinically distinguished from other central nervous system infections. Notably, of the cases of WN viral encephalitis diagnosed in NYC in 1999, approximately 40% of laboratory positive cases had severe muscle weakness; of these, 10 % developed flaccid paralysis with electromyographic findings consistent with an axonal neuropathy. This profound muscle weakness initially raised the possibility of botulism or Guillain-Barré syndrome.

patients with potential arboviral infections; 2) Implement laboratory-based surveillance for CSF specimens meeting sensitive but nonspecific criteria for arboviral infections (*e.g.*, mild to moderate pleocytosis and negative tests for the presence of non-arboviral agents such as bacteria, fungi, herpesviruses, and enteroviruses) and test them for evidence of WN virus infection.

(3) Special surveillance projects

Certain special projects may be used to enhance arboviral disease surveillance. Such projects include the Infectious Diseases Society of America Emerging Infections Network (IDSA EIN), Emergency Department Sentinel Network for Emerging Infections (EMERGEncy ID NET), Emerging Infections Programs (EIP) Unexplained Deaths and Critical Illnesses Surveillance, and the Global Emerging Infections Sentinel Network of the International Society of Travel Medicine (GeoSentinel). In some areas, syndromic surveillance systems are in place or being developed. The "piggy-backing" of surveillance for WN meningoencephalitis and milder clinical forms of WN fever, such as fever with rash or lymphadenopathy, onto existing syndromic surveillance systems, including those involving large health maintenance organizations, should be encouraged. Real-time computerized syndromic surveillance in emergency departments, and special surveillance projects to identify WN virus disease in pediatric populations, may be useful.

3. Specimens

a. Cerebrospinal fluid

As early as the first few days of illness, IgM antibody to WN virus can be demonstrated in CSF by antibody-capture ELISA. Virus also may be isolated, or detected by RT-PCR, 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 WN and other arboviruses by ELISA or neutralization tests. Although tests of a single acute-phase serum specimen can provide evidence of a recent WN virus infection, a negative acute-phase specimen is inadequate for ruling out such an infection, underscoring the importance of collecting paired samples. CDC will collect and distribute human WN virus antibody-positive control serum to state and local public health laboratories for use in serologic testing.

c. Tissues

When arboviral encephalitis is suspected in a patient who undergoes a brain biopsy or who dies, tissues (especially brain samples, including various regions

of the cortex, midbrain, and brainstem) and, in fatal cases, heart blood and buffy coat samples should be submitted to CDC or other specialized laboratories for arbovirus and other testing. Individual tissue specimens should be divided, and half should be frozen at -70°C and the other half placed 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 (also available at www.cdc.gov/epo/mmwr/preview/mmwrhtml/00047449.htm) should be used to classify cases as confirmed or probable, once appropriate laboratory results are available (also see Section IIA).[26]

C. Geography and Timing

1. Northeastern United States

   Active ecological surveillance and enhanced passive surveillance for human cases should begin in the early spring and continue through the fall until mosquito activity ceases because of cold weather. Surveillance in urban and surrounding areas should be emphasized.

2. Southern United States

   WN virus could conceivably be circulating in some areas throughout the year, especially the Gulf States. Active ecologic surveillance and enhanced passive surveillance for human cases should be conducted year round in these areas.

3. Western and Central United States

   Although there is currently no evidence for this, WN virus could spread to Central and South America by migratory birds. As these birds mingle and return north, the possibility exists that the virus could spread to the western and central U.S. There is also the potential for the virus to spread westward from the currently involved zone in the northeast and mid-Atlantic. Efforts to Increase awareness of the medical community, dead bird surveillance and enhanced passive human surveillance for WN virus should be initiated in the early spring.

4. Other Areas of the Western Hemisphere

   Development of surveillance systems capable of detecting WN virus activity should be encouraged in Canada, the Caribbean and Central and South America. WN virus 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 WN and SLE viruses, usually is clinically inapparent, or causes a non-specific viral syndrome in most patients. Definitive diagnosis, therefore, can only be made by laboratory testing using specific reagents. Active surveillance, to be successful, 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 WN virus, and details of how the laboratory diagnostic tests are used to support surveillance, are presented in Appendix B.

A. Biocontainment

1. Laboratory Safety Issues

a. WN virus is classified as a BSL3 agent by the Subcommittee on Arbovirus Laboratory Safety (SALS) of the American Committee on Arthropod-Borne Viruses, and CDC. Therefore, it is recommended that laboratory investigations that involve handling of infectious virus require BSL3 containment. Specifications for BSL3 containment are available in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories" (BMBL).[27] Web site links for the BMBL book http://bmbl.od.nih.gov and http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm are available. Concerns have been expressed that strict BSL3 containment for handling suspect human or animal specimens in the clinical diagnostic setting would severely limit the number of laboratories capable of detecting WN virus infections in a timely manner. The BMBL document specifically addresses this issue as follows:

"It is recognized, however, that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (*i.e.*, double door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (*e.g.*, diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing etc.) may be achieved in a Biosafety Level 2 facility, providing 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification should be made only by the laboratory director."

The following are some specific recommendations related to the implementation of the BSL2 modification.

(1) Handling of clinical material (serum, CSF, etc) under BSL2 containment should only be conducted in Class 2 biological safety cabinets that are located in laboratory rooms with restricted access.

(2) Handling and initial processing (homogenization) of field collected specimens (mosquito pools, tissues etc.) for nucleic acid analysis should only be conducted in Class 2 biological safety cabinets located in laboratory rooms with restricted access until virus infectivity has been destroyed (i.e., through the addition of a lysis buffer). At this stage, nucleic acid isolation can proceed under BSL2 conditions.

(3) Aerosol-producing procedures (*e.g.*, ELISA plate rinsing) should be performed in a Class 2 biological safety cabinet or using instruments which provide aerosol protection.

b. A protocol for field collection of dead birds and necropsy is being drafted by USGS for distribution. All bird necropsies should be done in a Class 2 biological safety cabinet.

2. Shipping of Agents

Shipping and transport of WN virus and clinical specimens should follow current International Air Transport Association and Department of Commerce recommendations. Because of the threat to the domestic animal population, a USDA shipping permit is required for transport of known WN virus isolates.

IATA dangerous goods website: http://www.iata.org/cargo/dg/

USDA-APHIS National Center for Import /Export: http://www.aphis.usda.gov/ncie/

B. Serologic Laboratory Diagnosis

Accurate interpretation of serologic findings requires knowledge of the specimen. For human specimens, it is important that the following data 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) current address and travel history in flavivirus-endemic areas; 5) history of prior vaccination against flavivirus disease (*e.g.*, yellow fever, Japanese encephalitis, or Central European encephalitis); and 6) brief clinical summary including suspected diagnosis (*e.g.*, encephalitis, aseptic meningitis).

1. Human

a. Since no commercial kit is available for human serologic diagnosis of WN virus infection, the CDC-defined IgM and IgG ELISA should be the front-line tests for serum and CSF.[28-30] These ELISA tests are the most sensitive screening assays available. The HI test may also be used to screen samples for flavivirus antibodies. Laboratories performing HI assays need be aware that the recombinant WN virus antigens produced to date are not useful in the HI test; mouse brain source antigen (available from CDC) must be used in HI tests.

b. To date, the prototype WN virus strains Eg101 or NY99 strains have performed equally well as antigens in diagnostic tests for WN virus 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 WN virus testing should participate in a proficiency testing program through experienced reference laboratories such as the DVBID in Fort Collins, CO, or the NVSL in Ames, IA.

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. To accommodate testing of other species, USDA, USGS and CDC will pursue the development of species-independent ELISA tests.

C. Virologic Laboratory Diagnosis

Experience gained in WN virus diagnostic testing over the past 2 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:

Clinically ill humans - CSF (serum samples may be useful early in infection)
Human (biopsy or postmortem) - brain tissue
Horses (postmortem) - brain tissue (including brainstem), spinal cord tissue
Birds - kidney, brain, heart
Other mammals - multiple tissues, especially kidney and brain

c. Confirmation of virus isolate identity can by accomplished by indirect immunofluorescence assay (IFA) using virus-specific monoclonal antibodies, nucleic acid detection or virus neutralization.

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 WN virus 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.

Nucleic acid detection methods including RT-PCR, TaqMan and nucleic acid sequence based amplification (NASBA) methods may be used to confirm virus isolates as WN virus.

Virus neutralization assays also may be used to differentiate viruses, by using four-fold or greater titer differences as the diagnostic criterion in paired specimens (acute and convalescent).

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 WN virus 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.[31, 32]

(2) A well-characterized antigen-capture ELISA is now available for detection of SLE[33, 34] and WN virus antigen (CDC, unpublished) in mosquito pools and avian tissues.

b. Nucleic acid analysis

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

(1) RT-PCR of tissues, mosquito pools, and CSF has proven to be a reliable method for use in surveillance. RT-nested PCR has reliably detected WN virus nucleic acid in equine brain and spinal cord tissues. Standardized protocols developed by reference laboratories should be disseminated. 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.[24, 35]

(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.

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 WN virus 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.[36] Programs consistent with best practices and community needs should be established at the local level and, at minimum, should be capable of performing surveillance adequate to detect WN virus epizootic transmission activity that has been associated with risk of disease in humans or domestic animals. Integrated mosquito management programs to minimize risk of WN virus 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 and the New Jersey Mosquito Control Association and the Florida Coordinating Council on Mosquito Control)[37-39]

A. Surveillance

Effective mosquito control begins with a 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 perform systematic surveillance for new sources. A mosquito identification specialist normally has the task of identifying the larvae to species. Properly trained mosquito identification specialists can separate mosquito nuisance and vector species. Responsible control programs target vector and pest populations for control and avoid managing habitat that supports benign species.

2. Adult Mosquito Surveillance

   Adult surveillance measures mosquito populations that have emerged from aquatic habitats. Various methods are available for this purpose and have been demonstrated to be effective in collecting certain mosquito species.[40] The New Jersey light trap, CDC miniature light trap, and other modifications of this design, with or without carbon dioxide bait, have been used extensively for collecting adult mosquitoes.[41] Gravid traps frequently are used to measure populations of *Culex pipiens* and *Culex restuans*, which have been incriminated as the primary enzootic vectors of WN virus in the northeastern states.[42, 43] Resting boxes frequently are used to measure populations of *Culiseta melanura*, a bird-feeding mosquito that is important in the amplification of EEE virus. Pigeon-baited traps are sometimes employed to measure *Culex* mosquitoes that amplify SLE virus. Trap deployment should address carefully species habitat requirements on several spatial scales.

3. Virus Surveillance

   The structure and function of virus surveillance in the vector population is described in more detail in section I.A.3. In general, the purpose of this component of the vector management program is to determine the proportion of the mosquito population carrying the virus, or the Minimum Infection Rate (MIR, expressed as the number infected per 1000 specimens tested). Specimens collected by the adult mosquito surveillance program, plus specimens collected in key areas that may provide important indicators of virus transmission activity and related human risk, can be used for this purpose. Mosquito collections made at permanent study sites can provide important baseline data to which current surveillance data are compared and decisions about human risk and need for emergency interventions are made. Surveillance assets should be deployed to monitor activity in rural, suburban and urban setting to detect initial amplification, spread and population risk, respectively.

B. Source Reduction

Source reduction is the alteration or elimination of mosquito larval habitat to prevent mosquitoes from breeding there. 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 the activities of humans 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 police 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:

   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 where impoundments can collect stormwater or rainwater and create freshwater mosquito problems which 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 approx. 3 ft. or less in depth rather than the deep ditching used in years past) is considered more environmentally acceptable because with shallow ditches, fewer unnatural hydrological impacts occur to the marsh.

C. Chemical Control

When source reduction and water management are not feasible, or have failed because of unavoidable or unanticipated problems, chemicals are used judiciously to control both adult and immature mosquito populations. In addition, chemical controls may be required to prevent disease when surveillance indicates the presence of infected adult mosquitoes poses a risk to health. The chemicals used by mosquito control agencies must comply with state and federal requirements. All pesticide applicators and operators in most states are required to be licensed or certified by the appropriate state agencies. Chemical treatments can be directed against either the immature or adult stage of the mosquito life cycle.[44]

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 are 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 "biorational" larvicides such as *Bacillus thuringiensis israelensis* (*Bti*, a bacterial larvicide), *Bacillus sphaericus*, and methoprene (Altosid, an insect growth regulator); and several oils (Golden Bear-petroleum based and Bonide-mineral based); and in some limited habitats diflubenzuron (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. Important goals when applying larvicides are that the material should be specific for mosquitoes, minimize impacts to non-target organisms and must, in many instances, be capable of penetrating dense vegetation canopies. Larvicide formulations (*e.g.*, 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 result in the emergence of a large mosquito brood resulting in the need for broad-scale adulticiding.

2. Adulticiding

   Adulticiding, the application of chemicals to kill adult mosquitoes by ground or aerial applications, is usually the least efficient mosquito control technique. Nevertheless, adulticiding based on surveillance data is an extremely important part of any integrated mosquito management program. 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.[45-49] Ground or aerial applied thermal applications of adulticides also are 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 a property border can provide some control benefits. Mosquito adulticiding differs fundamentally from efforts 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 pose minimum risks to human health and the environment when used according to the label.[37] Adulticides labeled for mosquito control include several organophosphates such as malathion and naled. Some natural pyrethrins, synthetic pyrethroids (permethrin, resmethrin and sumithrin) also hold adulticide labels. Insecticide selection and time of application should be based on the distribution and behavior of the target mosquito species. Most *Culex* are nocturnal, compromising aerial application in urban areas.

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).[38] Ideally, this includes 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 the causes of failure of disease control; 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.[50, 51] The techniques regularly used are:

1. Management by Moderation - preventing onset of resistance by:

    a. Using dosages no lower than the lowest label rate to avoid genetic selection.
    b. Using less frequent applications.
    c. Using chemicals of short environmental persistence.
    d. Avoiding slow-release formulations.
    e. Avoiding the use of the same class of insecticide to control both adults and immature stages.
    f. Applying locally -- Currently, most districts treat only hot spots. Area-wide treatments are used only during public health alerts or outbreaks.
    g. Leaving certain generations, population segments or areas untreated.
    h. Establishing high pest mosquito densities or action thresholds prior to insecticide application.
    i Alternation of biorational larvicides and IGRs annually or at longer intervals.

2. Management by Continued Suppression - a strategy used in areas of high-value (*e.g.*, heavy tourist areas in the case of mosquito control) or where insect 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, no resistance will occur because homozygous- resistant individuals do not exist or they are at such a small frequency that quick population build-up is unlikely. 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).

3. 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 (*e.g.*, 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 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 semi-permanent water bodies where mosquito larvae occur, providing some measure of control. Other biocontrol agents which have been tested for use by mosquito control, but 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.

F. Continuing Education

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. Community Outreach and Public Education

Public education is directed toward the general public to teach mosquito biology and encourage citizens to utilize prevention techniques. Examples include: fact sheets and brochures, classroom lectures at schools, slide shows, films and videos on mosquitoes and their control, and exhibits at fairs. It is important that the effectiveness of the techniques selected be tested prior to use and evaluated after implementation to determine if they were effective in increasing public knowledge and altering attitudes and behaviors. Obtaining the interest and investment of the community is critical to public education and outreach programs. Developing a community task force that includes civic, business, health, and environmental concerns has proven valuable in education programs, and in developing a common message. Additional assistance can be obtained from local media contacts and topical experts from local or state health departments, Centers for Disease Control and Prevention, and the American Mosquito Control Association.[52, 53]

H. 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 should can contact state mosquito control associations to provide examples of enabling legislation.

I. Vector Management in Public Health Emergencies

Epidemic or epizootic transmission of enzootic arboviruses typically precedes detection of human cases by several days to two weeks or longer (*e.g.*, as found in SLE epidemics).[54, 55] Therefore, a surveillance program adequate to monitor WN virus transmission activity levels that indicate human risk must be in place. Control activity should be initiated in response to evidence of virus transmission, as deemed necessary by the local health departments. Such programs minimally should consist of an intervention program including public education emphasizing personal protection and residential source reduction; municipal larval control to prevent re-population 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 control efficacy.

As evidence of sustained or intensified virus transmission in an area increases, emergency preparations should be commenced and implemented as needed. This is particularly important in areas where vector surveillance indicates that potential accessory vectors (*e.g.*, those demonstrating mammalophagic host ranges) are infected with WN virus. Delaying adulticide applications in areas with these surveillance indicators until human cases occur negates the value and purpose of the surveillance system.

J. 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, truck application can be done throughout the night, thereby targeting night-active mosquito species. Ground 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 ground-based applications. 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, 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 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. An emergency response plan developed for the city of New Orleans, Louisiana[44] 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 *Culex 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 the virus. 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 applications timed appropriately. 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.

K. Determining the Scope of Mosquito Adulticiding Operations

Once arbovirus activity is detected in a jurisdiction and a decision is made to implement 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) determines 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 WN virus. 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 risk of disease. At a minimum, the following factors must be considered when deciding the scope of the adulticiding effort:

1. The general ecology of the area—key habitat types, and the presence of natural barriers such as large rivers;

2. The flight range of affected/infected bird species;

3. The flight range of vectors known or believed to be of importance in the area;

4. The population density and age (proportion of parous females) of the vectors;

5. The length of time since birds started dying or became infected in the impacted area (typically, there may be a lag of several weeks between recovery of dead birds and confirmation of WN virus infection) or since virus-positive mosquito pools were collected;

6. The human population at risk—distribution relative to the positive locality (*e.g.*, urban vs. rural), community perception of the relative risk of pesticides vs. WN virus infection, age demographics of the area;

7. Evidence of persistent transmission activity detected by the surveillance program;

8. Season of the year - how much time the transmission risk can be expected to persist until the vector(s) enter diapause.

Several of these factors will be unknown or only poorly known. 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 large human population densities.

L. Evaluation of Adult Mosquito Control

The following parameters should be periodically monitored during control operations:

1. Minimum requirements:

   a. Pre and post spray mosquito densities inside and outside control area using CO_2-baited traps and gravid traps.

   b. Mosquito infection rates pre and post spray inside and outside control area.

   c. Weather conditions during application (temperature, wind speed, direction).

2. Desirable additions if capacity exists:

   a. Population age structure of key mosquito species (*Cx. pipiens*).

3. In addition, the following should be documented for each piece of application equipment:

   a. Droplet size of ULV.

   b. Flow rate.

4. During application, GPS monitoring of spray track should be conducted if equipment is available on aircraft.

M. Public Information Programs

Public acceptance will be critical for emergency adult mosquito control to happen, especially in areas were mosquito control is an unfamiliar activity. Public education programs to distribute information about the nature of mosquito-borne disease, and the risks and benefits of adulticide use will be necessary. Public information offices at federal, state and local levels need to be involved in this process. Repeated efforts will

be needed regarding core messages about personal protection and source reduction. The media will significantly influence the public's perception of emergency adulticiding and adequate public health information resources will be needed to assure the government's rationale is well represented. Several public information resources are currently available through the EPA and CDC. These materials should be incorporated into routine press releases throughout the season and augmented in the event that adulticiding activities are required.

N. Guidelines for a Phased Response to WN Virus Surveillance Data

The principle goal is to minimize the health impact of the WN virus in humans, as well as in domestic and zoo animals. Given the limited understanding of the ecology and epidemiology of the WN virus in the U.S., the sporadic nature of the occurrence of arboviral encephalitis, and the limitations of prevention methods, it is expected that prevention and control measures, no matter how intensive, cannot prevent all WN virus infections in humans.

The recommended response levels for the prevention and control of the WN virus should augment, but not replace long standing mosquito control efforts by established mosquito control programs. These programs often have two objectives: 1) to control nuisance mosquitoes, and 2) to control vector mosquitoes that can transmit pathogenic organisms. Nuisance mosquito control often has different objectives than vector control, and the mosquito species to be controlled are often different than vector species. Established mosquito control programs often have a long-standing experience with the surveillance and control of the other established arboviral encephalitis viruses found in the U.S. These programs have established thresholds for response based on years of data. No such long-standing experience exists for the WN virus. Therefore, the recommendations for WN virus must be interpreted only in light of established practices for the other established arboviral encephalitis virus control programs.

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

1. *All of the continental states should prepare for the occurrence of the WN virus.* The WN virus epizootic expanded markedly in 2000. Given its occurrence in many different habitats and ecosystems in the Old World, and the fact that the SLE virus, a related flavivirus, is widespread in the U.S., suggests the potential for additional geographic spread of the WN virus. The kinds of preparation may vary with the proximity to the known spread of the virus in 2000. At a minimum, a plan for the surveillance, prevention, and control of the WN virus should be developed.

2. *Measures of the intensity of the WN virus epizootic in an area should be considered when determining the level of the public health response.* Although only one year of prospective data are available, analyses indicate that the WN virus epizootic intensity as measured by avian mortality, such the number of dead crows found per square mile, may indicate increased human infection risk. The minimum infection rate in *Culex* mosquitoes, the number of infected mosquito species in an area, and the WN virus antibody prevalence in hatching-year live birds may also portend

increased human risk, although these data are limited. Data from NYC indicated that isolated cases of WN infection in humans were more likely in counties with >0.1 dead crow reports per square mile per week and in Staten Island, the only location with a human outbreak in 2000, the levels exceeded 1.5 dead crow reports per square mile per week. These figures should be interpreted as a guide, rather than absolute, because the human cases in 2000 were limited to smaller urban counties in and around the NYC metropolitan area. It is unknown what levels of epizootic activity will correlate with increased human risk in subsequent years, in other regions of the country, and in more rural areas.

3. *Flexibility is required when implementing the guidelines*. Knowledge gained from subsequent surveillance and research data are likely to change the recommendations for response. Specific recommendations that will fit all possible scenarios also cannot be made, particularly at a local level. Therefore, public health action should depend on interpretation of the best available surveillance data in an area, in light of these general guidelines. In addition, many other 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 transmission (surveillance events earlier in the transmission season will generally have greater significance),

    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 WN virus surveillance data are indicated in the table below. Local and regional characteristics may alter the risk level at which specific actions must be taken.

Table 1. Suggested Guidelines for Phased Response to West Nile Virus Surveillance Data

Risk category	Probability of human outbreak	Definition	Recommended response*
0	None	Off-season; adult vectors inactive; climate unsuitable.	Develop WN virus response plan. Secure surveillance and control resources necessary to enable emergency response. Initiate community outreach and public education programs.
1a	Remote	Spring, summer, or fall; areas unlikely to have WN virus epizootic in 2001 based on lack of previous or current WN virus activity in the region.	Response as in category 0, plus: Conduct entomologic survey (inventory and map mosquito populations; see AMCA and other manuals for guidance); community outreach and public education; avian mortality, human encephalitis/meningitis and equine surveillance.
1b	Remote	Spring, summer, or fall; areas anticipating WN virus epizootic in 2001 based on previous or current WN virus activity in the region; no current surveillance findings indicating WN virus epizootic activity in the area.	Response as in category 1a, plus: 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; public education emphasizing source reduction.
2	Low	Spring, summer, or fall; areas with initial, sporadic or limited WN virus epizootic activity in birds and/or mosquitoes.	Response as in category 1b, plus: Increase larval control and 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). Consider focal or targeted adult mosquito control if surveillance indicates likely potential for human risk to increase.
3	Moderate	Spring, summer, or fall; areas with initial confirmation of WN virus in a horse and/or a human, or moderate WN virus activity in birds and/or mosquitoes.	Response as in category 2, plus: Strongly consider adult mosquito control if surveillance indicates likely potential for human risk to persist or increase.
4	High	Spring, summer, or fall; quantitative measures indicating WN virus epizootic activity at a level suggesting high risk of human infection (for example, high dead bird densities, 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) and abundant adult vectors.	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); initiate or continue active surveillance for human cases; implement adult mosquito control program targeted at areas of potential human risk.
5	Outbreak in progress	Multiple confirmed cases in humans; conditions favoring continued transmission to humans (see level 3)	Response as in category 4, plus: Implement or intensify emergency adult mosquito control program, enhanced risk communication about adult mosquito control, monitor efficacy of spraying on target mosquito populations. If outbreak is widespread and covers multiple jurisdictions, consider wide-spread aerial spraying as per the WN virus Emergency Contingency Plan.

* 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 lower 48 contiguous 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 1) the health risk of arboviral diseases in the area and 2) 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 complement 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. In many health departments, a chronic shortage or complete absence of medical entomologists exists. Addressing this deficiency should be a high priority. Many jurisdictions also have a shortage of expertise in wildlife pathobiology, which should also be addressed. In the event of an arboviral 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 to 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 WN Virus Infections

It is important to distinguish between increasing long-term laboratory capacity, and increasing short-term capacity in the wake of the 1999/2000 epidemics. The former is preferred and should be emphasized over the latter. Laboratories with an existing capability for arbovirus serology should consider adding serologic screening tests for WN virus to their repertoire. For serologic screening of patients and

mosquito pools, arrangements can be made with CDC to transfer existing ELISA 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 SLE, EEE, WEE, 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 health public health agencies are responsible for assuring provision of preventive activities to reduce the risk of WN virus infection to individuals residing in their jurisdictions. This includes provision of community-wide education to foster activities by individuals to reduce mosquito breeding and to take 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 recommendations on the indications and decisions concerning mosquito adulticiding. Education, communication and maintaining local media contacts are generally primary functions of the local public health agency. Included in this responsibility is risk communication 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 assured).

1. Assessment of risk-- based on surveillance data (including mosquito, bird, and human surveillance). Surveillance data may also include reports from individuals or health care 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.[52, 53]

3. Communication with the media.

4. Development of a preventive plan including education, mosquito breeding control 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 outbreaks of WN virus involves all levels of government including the federal governments of the U.S., 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 to share 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 are needed.

During an epidemic involving multiple jursidictions, CDC and appropriate, authorized users will use Epi-X, a CDC-based system for secured electronic communication or similar integrated communication systems for rapid dissemination of information on public health events of public health significance. User groups should be constructed in a logical and efficient manner. For example, some public health officials need to receive veterinary and wildlife data routinely, whereas others do not; the converse is also true.

Geographic information system (GIS) data should be used to track epidemics spatially.

A. Human Epidemiological, Clinical and Laboratory Data Collection

CDC will provide generic templates for electronic databases that can be rapidly customized and stored centrally to allow efficient and secure interjurisdictional sharing of human clinical and laboratory data during epidemics. Issues include:

1. Efficiency and Integrity

   Centralized electronic databases should be designed to balance the need to maintain data integrity with the desire to minimize duplicate data entry. On a regular and frequent basis, such centralized databases should be backed up automatically with at least one recent backup copy maintained off site.

2. Confidentiality and Security

   Patient confidentiality statutes vary from state to state. Data can be shared between jurisdictions if recipients agree to adhere to the confidentiality statutes of the state providing the data. Electronic databases should be appropriately secured

by passwords, to limit access and minimize opportunities for breaches in confidentiality or security.

3. Standardization of Data Collection Instruments

   Ideally, during an epidemic involving multiple jurisdictions, data collection (by both electronic and written means) should be done in a standardized fashion across all jurisdictions. While a more integrated and National Electronic Disease Surveillance System (NEDSS)-compliant (http://www.cdc.gov/od.nissb/docs.htm) data collection instrument is developed and disseminated, CDC will provide a list of the characteristics of data variables (*i.e.*, types, names, lengths, and order) that are contained in centralized electronic databases**.**

4. Centralization

   During an epidemic, centralized electronic databases for sharing epidemiological, clinical and laboratory information should be maintained at CDC. These databases should be accessible to authorized users via the Internet after all personal identifying information has been removed.

B. National Reporting of Human Cases of WN Encephalitis

WN encephalitis is not on the list of nationally notifiable diseases maintained by the Council of State and Territorial Epidemiologists (CSTE) in consultation with CDC. However, this does not preclude states from reporting such cases to CDC, and CDC has designated 10056 as a specific disease code ("EVENT" code) for use in reporting WN encephalitis 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 for classifying cases as either confirmed or probable.[(Ref 9)] WN meningoencephalitis should be added to the nationally notifiable diseases list.

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, in terms of the latter, patient confidentiality is generally not an issue, except for owned animals, while standardization of data collection is a far more challenging issue because of the relatively large number of species often being studied. Specific needs include:

1. Accurate Taxonomic Identification of Specimens

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

2. Unique Identification (UI) Numbering System for Specimens

   A standardized UI numbering system should be developed (or adopted from an existing system) for wide-scale use by each state or independent jurisdiction. The numbering system should readily distinguish between each major animal group (*i.e.*, humans, birds, and mosquitoes), county or township of collection, the year of collection, and a specimen-specific number.

3. Durable Tagging System for Field-Collected Specimens

   It is critical that field specimens--whether blood, tissues, or whole animals--be properly labeled so that specimen identification will not be lost during shipment to testing facilities.

4. Standardized Data Collection and Specimen Submission Instruments

   Standardized data collection forms should be developed and used for birds, mosquitoes, and other animals. Some instruments already exist (*e.g.*, at the USGS's National Wildlife Health Center and at CDC) and these could be a starting point for development of additional instruments for general or specific usage. A difficulty may be the wide taxonomic range (*e.g.*, from mosquitoes to large mammals) and large number of species often studied.

VI. Research Priorities

The human and animal health implications of the introduction of WN virus to the U.S. and to the Western Hemisphere are unknown at this time. 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 WN Virus

   To determine the geographic distribution of WN virus in the Western Hemisphere, existing laboratory-based surveillance systems for WN virus 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 WN Virus Dispersal

   Experience in Europe and the Middle East suggests that WN virus regularly is introduced to new geographic areas along bird migration routes.[1,2] 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

Very little is known about the vertebrate host and mosquito vector relationships of WN virus 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 WN virus will be maintained in the U.S. Overwintering mechanisms in *Culex* and *Aedes* species mosquitoes 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

Currently, effective prevention and control of WN virus can only be accomplished by mosquito control. It is critical that we have a better understanding of the principal mosquito vectors involved in maintenance, bridge (from enzootic to peridomestic), and epidemic/epizootic transmission. Different vector species may be important in each geographic or ecologic region. Understanding their biology and behavior will allow more effective surveillance and development of targeted control methods.

F. Development and Evaluation of Prevention Strategies

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

A very long-term goal of is the derivation 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 WN virus threat and of the actions needed to reduce exposure to virus-infected mosquitoes. These actions include using mosquito repellent, reducing mosquito breeding sites around the home and wearing long-sleeved shirts and pants when going outside into mosquito-infested areas. The success of these public information campaigns have not been formally evaluated using scientific instruments such as knowledge and behavior surveys. The cost of these public awareness campaigns is high, so formal attempts to assess their success is needed.

G. Laboratory Diagnosis

Surveillance for WN virus will require accurate laboratory diagnostic tests. Ideally, these tests will be simple and inexpensive, and will distinguish between WN virus 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 WN virus 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 WN virus infection.

I. Risk Factor Studies

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

J. Viral Pathogenesis

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

K. Genetic Relationships and Molecular Basis of Virulence

Only since 1996 has WN virus 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.

L. 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.

M. Antiviral Therapy for WN Virus and Other Flaviviruses

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

N. The Economic Cost of the Northeastern WN Virus Epidemic/Epizootic

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

O. WN Virus Impact on Wildlife

WN virus has the potential to make major impacts on 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 (*e.g.*, Corvidae). Research is needed to analyze and define these impacts to determine if the development of new epizootic intervention strategies is needed. Research is also needed to determine what long-term effects WN virus infection may have on its animal hosts.

P. Evaluation of Pesticide Exposure of Humans

Throughout the country, local public health officials will have to decide what mosquito control measures they will employ to prevent human infection with WN virus. Central to this issue is the selection of appropriate larviciding and adulticiding agents. It is necessary to collect clear and unambiguous information on what negative effects these applications may have on human health. These data can be used to support scientifically the selection of mosquito control methods and to convey this information to the public.

Q. Continue Attempts to Identify the Method of WN Virus Introduction into the U.S.

Even though it may never be determined how WN virus was first introduced into the U.S., a better understanding of this event will be useful in planning for and evaluating future introductions of new disease agents.

Appendix A – Agenda and List of Meeting Participants

P R O G R A M

WEDNESDAY, JANUARY 31

6:00 - 8:00 PM **Early Registration**

8:00 PM **Overview of Arbovirus Ecology** Robert Craven
Chester Moore
Nicholas Komar

(*Optional attendance; primer for those wanting an overview of arboviral disease surveillance, prevention, and control*)

THURSDAY, FEBRUARY 1

(All Plenary Sessions will be held in the Omni Grand Ballroom. Continental Breakfast and Breaks will be in the Grand Ballroom Foyer)

7:30 - 9:30 AM **Registration**

7:30 - 8:30 AM **Continental Breakfast**

8:30 AM **Welcome and Call to Order**
Moderator: Duane J. Gubler

Opening Remarks
- William Smith, National Association of County and City Health Officials
- Katherine Kelley, Association of Public Health Laboratories
- Matthew Cartter, Council of State and Territorial Epidemiologists

Charge to Participants — Duane J. Gubler

West Nile Virus: The U.S. Experience — John Roehrig

10:00 AM **Break**

Module 1: Surveillance

10:30 AM **1A: Bird-based Surveillance for West Nile Virus**
Co-chairs: Nicholas Komar, Bob McLean

10:30 - 11:00 AM **Presentations**
- Dead Bird Surveillance – A National Perspective: Bob McLean

- Dead Bird Surveillance – The State and Local Perspective: Millie Eidson
- Sentinel Live Bird Surveillance: Nicholas Komar

11:00 - 11:30 AM **Panel Discussion:** Ward Stone, Tracy McNamara, Leonard Marcus, Randy Nelson, Jonathan Day

Topics

- Integration of wildlife and public health agencies for surveillance
- Integration of zoos into public health surveillance
- Increasing the value of live bird sentinels
- Increasing the value of dead bird surveillance
- Selection of target species
- Triage protocols (is necropsy necessary?)
- Trigger criteria for decision-making
- Significance of a single dead bird

11:30 - 12:30 PM **General Discussion**

12:30 PM **Lunch**

1:45 PM **1B: Mosquito Trapping, Identification, Pooling, and Testing**
Co-chairs: Harry Savage, Dennis White

1:45 - 2:15 PM **Presentations**

- Virus Isolation and Vector Identification, Importance to West Nile Virus Surveillance and Control: Harry Savage
- Public Health Risk Assessment: Dennis White
- The Staten Island Experience, 2000: Varuni Kulasekera

2:15 - 2:45 PM **Panel Discussion:** Wayne Crans, L.A. Williams, Bruce Harrison, Theodore Andreadis, Vicki Kramer

Topics

- What was learned from mosquito surveillance in 2000 and how will this information impact future programs?
- How will the geographical location of trap sites be determined? (Site targeted, random, uniform?)
- Will sites be fixed or flexible?
- What traps will be used and what species will be targeted?
- How will the quality of mosquito identification be improved?
- What virus detection methods will be used?
- How can the efficiency of mosquito trapping and testing be improved to better predict increasing risks for human disease?
- What resources should be applied to determine vectorial status in order to focus intervention efforts? (Increase

coverage of mosquito taxa tested for virus, bloodmeal analysis, comparative vector competence)

2:45 - 3:15 PM **General Discussion**

3:15 PM **Break**

3:30 PM **1C: Humans, Equines, and Other Mammals**
Co-chairs: Roy Campbell, Randall Crom

3:30 - 4:00 PM **Presentations**

- Surveillance for West Nile Virus Disease in Humans: Marci Layton
- Surveillance for West Nile Virus Disease in Equines: Randy Crom
- Surveillance for West Nile Virus Disease in Other Mammals: Millie Eidson

4:00 - 4:30 PM **Panel Discussion:** Matt Cartter, Suzanne Jenkins, Clifford Johnson, Martin Levy, Godwin Obiri

Topics

- What are the major problems with the existing surveillance systems? What are their potential solutions?
- Are additional types of surveillance needed (e.g., to determine the effects of West Nile virus disease on small wild mammals)?
- Are changes in the current surveillance case definitions needed?
- Are additional serosurveys of human, equine, and/or other mammalian populations needed?

4:30 - 5:30 PM **General Discussion**

5:30 PM **Adjourn**

7:00 PM **Meeting of Work Groups**

- 1A: Bird-based Surveillance — Poplar Room
- 1B: Mosquito Trapping — Juniper Room
- 1C: Humans, Equines, Other Mammals — Dogwood Room

FRIDAY, FEBRUARY 2

7:30 - 8:30 AM **Continental Breakfast**

Module 1: Surveillance (continued)

8:30 AM **1D: Data Collection and Sharing**
Co-chairs: Anthony Marfin, Perry Smith, John Loonsk

8:30 - 9:20 AM **Presentations**

- Submission of Surveillance Data in 2000: Anthony Marfin
- Modifying the Methods Used to Collect West Nile Surveillance Data in 2001: Perry Smith
- New IT Solutions for Moving Data from States to CDC: John Loonsk
- IT Solutions for Collecting Data in Counties and Moving Data to the State: Ivan Gotham
- Data Verification, Secured Correspondence Systems, and Other Issues of Inter-jurisdictional Data Sharing: Lyle Petersen

9:20 - 9:40 AM **Panel Discussion:** Bela Matyas, James Miller

Topics

- What other methods can be used to reduce the current resources that are necessary to collect, submit, and process surveillance specimens at the county and state levels?
- Is there a sensible and representative specimen collection and testing algorithm that can be used in counties and states to reduce the burden of surveillance?
- What other methods may be considered to reduce hand-entry of data at the county, state, or federal level?
- What level of data verification should be used prior to dissemination of information?
- What methods can be used for public health officials in one state to rapidly and effectively notify their neighboring states, or is this a function of CDC?
- Should all arboviral infections be reported to and shared by Arbo-NET in the same fashion as West Nile virus reporting was in 2000?

9:40 - 10:00 AM **General Discussion**

10:00 AM **Break**

Module 2: Prevention and Control

10:30 AM **2A: Linking Surveillance to Prevention**
Co-chairs: Lyle Petersen, James Hadler

10:30 - 11:00 AM **Presentations**

- Surveillance Indicators for Predicting West Nile Viral Illness in Humans in New York City and New Jersey in 2000, and Prevention Measures for Responding to Them: James Miller
- Lessons from St. Louis Encephalitis Surveillance: Lisa Conti

11:00 - 11:30 AM **Panel Discussion:** Perry Smith, James Miller, Eddy Bresnitz,

Matt Cartter, Bela Matyas

Topics

- What are you trying to prevent in your jurisdiction (e.g., human infections, single cases of severe human disease, outbreaks of severe human disease, equine disease)?
- What were the pre-season plans in your jurisdiction for linking surveillance to prevention, and how did these plans function during the 2000 transmission season? If they changed during the season, what factors made you change them?
- How do you plan to respond to the following surveillance findings in 2001:
  - Positive bird(s)
  - Positive mosquito pool(s)
  - Horses or other mammals with West Nile virus morbidity/mortality
  - Humans with West Nile virus morbidity/mortality

11:30 - 12:30 PM **General Discussion**

12:30 PM **Lunch**

1:30 PM **2B: Integrated Pest Management Strategies**
Co-chairs: Roger Nasci, Kevin Sweeney

1:30 - 2:00 PM **Presentations**

- New York City's West Nile Virus Vector Management Programs: James R. Miller
- New Jersey's West Nile Virus Vector Management Program: Bob Kent
- Advances in ULV Technology: Droplets, Drift, and Deposition: James Dukes

2:00 - 2:30 PM **Panel Discussion:** David Dame, Ray Parsons, Peter Connelly, Dominick Ninivaggi

Topics

- What are the relative roles of larval population management and adult population management in a West Nile virus integrated management program?
- How best can urban West Nile virus vectors be managed?
- How do we best address sensitive habitats (e.g., wetlands) in West Nile virus vector management programs?
- What new information (research) is needed to optimize West Nile virus vector management?

- What application technologies and/or formulations perform better in urban settings?

2:30 - 3:15 PM **General Discussion**

3:15 PM **Break**

3:30 - 4:00 PM **2C: Pesticide Toxicity**

Presentations:
- Insecticide Application for Control of West Nile Virus Vectors: Pesticide Risk and Exposure: Kevin Sweeney
- Evaluating Exposure and Illness Related to the Use of Adulticides for Mosquito Control: Jessica Leighton

4:00 PM **2D: Evaluation of Prevention Programs**
Co-chairs: Chester Moore, David Dame, Bob Kent

4:00 - 4:30 PM **Presentations**
- Criteria for Evaluating Prevention Adulticiding Programs: Ray Parsons
- Legal Aspects of Program Evaluation: L. A. Williams
- Criteria for Evaluating Larviciding and Source Reduction Programs: Judy Hansen

4:30 - 4:50 PM **Panel Discussion:** Marc Slaff, Alice Anderson, Mark Latham

Topics
- How effective is mosquito control? How do you know?
- What are the minimal and the ideal evaluation criteria for control programs? Do these vary from state to state?
- How would you evaluate the effectiveness of "non-intervention" strategies (e.g., public education, use of repellents, re-scheduling of outdoor events)?
- Can control evaluation data be used to inform the community? How?

4:50 - 5:30 PM **General Discussion**

5:30 PM **Adjourn**

6:00 PM **Demonstration of CDC-developed Software for Reporting Surveillance Data** Grand Ballroom

7:00 PM **Meeting of Work Groups**

- Data Collection, Sharing Dogwood Room

- Linking Surveillance to Prevention Magnolia Room
- Integrated Pest ManagementJuniper Room
- Evaluation of Prevention Programs Pine Room

SATURDAY, FEBRUARY 3

7:30 - 8:30 AM **Continental Breakfast**

Module 3: Ecology and Biology

8:30 AM **Prospects for the Future -- Lessons Learned and Future Research Needs**
Co-chairs: John Roehrig, James Meegan

8:30 - 9:00 AM **Presentations**
- Field Biology: Roger Nasci
- West Nile Virus and Flavivirus Pathogenesis: Thomas Chambers
- West Nile Virus Vaccines and Therapy: Thomas Monath

9:00 - 9:25 AM **Panel Discussion:** Barry Beaty, Ian Lipkin, Robert Tesh, Andy Spielman

Topics:
- What are the important ecological research questions that need to be answered regarding natural West Nile virus transmission in the United States? How should they be addressed?
- How should we approach ecological research questions regarding the possible establishment of West Nile virus in epizootic foci in Central and South America?
- How does the NY99 strain of West Nile virus compare with Old World West Nile viruses with respect to virulence, pathogenesis, transmission, and host range?
- Is development of a West Nile virus vaccine feasible, practical, and desirable?
- What are current scientifically validated approaches to flavivirus-specific therapy? What new approaches should be considered?

9:25 - 10:15 AM **General Discussion**

10:15 AM **Break**

Module 4: Diagnostics and Virology

10:30 AM **Diagnostics: Virology, Serology, Biocontainment**
Co-chairs: Robert Lanciotti, Eileen Ostlund

10:30 - 11:00 AM **Presentations**

- Serologic and Virologic Tests for West Nile Virus: Robert Lanciotti
- West Nile Virus Testing in New York, 2000: Laura Kramer
- Veterinary Testing: Eileen Ostlund

11:00 - 11:30 AM **Panel Discussion:** Amy Glaser, Bob Myers, George Ludwig, John Roehrig, Susan Wong

Topics

- For maximum sensitivity, what tissue(s) should be tested in virus detection assays (PCR & IFA) from avians and equines? What other assays could be used for tissue testing: immunohistochemistry, in-situ hybridization? PCR on formalin-fixed tissues? Smears or frozen sections?
- What are the most definitive confirmatory serologic tests? What serologic tests should be developed for antibody detection in other species? Competition ELISA?
- What protocols, control reagents/validation reagents are available to laboratories embarking on West Nile virus serology testing (both human and veterinary testing)? Are there issues with inter-laboratory "harmonization" of results? What should be the biosafety precautions with respect to sample handling and testing at laboratories?

11:30 - 12:15 PM **General Discussion**

12:15 PM **Lunch**

Module 5: Public Health Infrastructure

1:30 PM **Co-chairs: Robert Craven, Mel Fernandez, Katherine Kelley**

1:30 - 2:00 PM **Presentations**

- Start-up of an Arbovirus Program in New York State: Dale Morse
- Establishing a Mosquito Control Program: David Dame

2:00 - 2:30 PM **Panel Discussion:** Lloyd Novick, Robert England, Patricia Hegadorn, Jody Henry Hershey, Stephen Capowski

Topics

- How can we develop local interagency cooperation to deal with a West Nile virus epidemic?
- How do we maximize communication with the state and with the public?
- How do we approach setting up an arbovirus surveillance program for likely agents?

2:30 - 3:00 PM **General Discussion**

3:00 PM **Break**

3:30 PM	**Meeting of Work Groups**	
	· Prospects for the Future	Pomodoro Room
	· Diagnostics and Virology	Juniper Room
	· Public Health Infrastructure	Willow Room

SUNDAY, FEBRUARY 4

8:30 – 9:00 AM	**Continental Breakfast**
9:00 AM	**Recommendations from Work Groups** **Moderator: Duane J. Gubler**
11:30 AM	**Meeting Closure**

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Appendix B: National West Nile Virus Surveillance System

Objectives:

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

- Monitor the geographic and temporal spread of WN virus in the U.S.
- Develop national public health strategies for WN virus 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 WN virus infections in humans and animals has been repeatedly identified as a high priority by states affected by WN virus in 1999-2000 and those that have a high potential for being affected in the future – states along the Atlantic and Gulf Coasts and in the Mississippi and Ohio River Valleys. However, all states conducting surveillance for WN virus and other arboviruses are encouraged to participate in this program to collect surveillance data. While the needs of individual jurisdictions vary, national WN virus surveillance should, at a minimum, focus on collection of data from:

- Mosquito surveillance
- Avian morbidity/mortality and infection surveillance
- Human surveillance

In addition to data from the states, data from commercial laboratories will be sought. This may provide a crude measure of trends in the incidence of the clinical syndromes of viral encephalitis and meningitis. 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, 2) provide them with a list of state health department contact persons, 3) regularly contact them to encourage reporting, 4) remind them of the need to have all positive screening tests for arboviral infections confirmed by state public health reference laboratories or CDC, and 5) request that they voluntarily and regularly report to CDC the number of patients tested for WN virus infection and other domestic arboviral infections by state. 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 new individuals or groups of individuals reported to, sampled by, or tested by a state's WN virus surveillance system, by county or similar jurisdiction within a state.

- *"Numerator"* data

  Definition: Detailed information on individual mosquito pools, sentinel species, dead birds, and ill humans, horses, or other species with confirmed or suspected WN virus infections, as determined by laboratory-confirmatory, -probable, or -equivocal 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 onto a database on a secured CDC web site, or other means (*e.g.*, importation of delimited records in ASCII format). Denominator data variables that will be collected are specified in Table 1. An appropriate submission schedule will be arranged by CDC, USGS, and the states submitting surveillance data via file uploading. For agencies uploading data files to CDC, the frequency of submissions will not be more than once a week. In addition,

- CDC will distribute the necessary software and provide the adequate licenses that will allow weekly 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, one each for mosquito, sentinel species, avian mortality, veterinary (non-avian), and human surveillance data.

- The system will accommodate updates and corrections of previously transmitted data by states.

- Following the entry of a week's data into the database at the state level, transmission of the data file to via CDC SDN will involve a minimal number of keystrokes. Security will be insured by use of the sender's "digital signature". CDC will arrange for those who will be transmitting surveillance data to CDC to obtain digital signatures.

- 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 National Atlas/USGS in Reston, Virginia.

- Using these data, reports will be generated automatically each week. Maps will be generated by CDC and by the USGS/National Atlas Project staff and available on the National Atlas web site. A basic set of dynamic maps and corresponding graphs and tables will be available weekly. The CDC web site and Epi-X (or a similar secured communication network) will contain links to the appropriate page(s) of the National Atlas web site.

Reporting "numerator" data:

CDC strongly encourages prompt ("real-time") reporting by telephone, electronic mail, FAX, or data entry into a web-based database. Reports should include all results, findings, and updates of potential public health importance (*e.g.*, reports of all human infections, reports indicating suspected, probable, or confirmed WN virus activity in animals in new areas). Because of confidentiality concerns, reports of suspected, probable, or confirmed human infections with WN virus should only be made by telephone report to CDC in Fort Collins, Colorado.

CDC staff will collect such reports in a standardized manner, allowing them to monitor 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 WN virus-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. WN virus laboratory and surveillance case criteria are specified in Table 3.

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

- Web-based data entry to a CDC server in Atlanta;

- Use of state-based, CDC-distributed, Access-based data entry/management software (Arbo-NET) with continuous file upload to a CDC server in Atlanta;

- Data messaging from a unique data collection system to a CDC server in Atlanta; or

- Call, FAX, or e-mail CDC-DVBID staff in Fort Collins, Colorado.

  In the first three methods, all data entry is done by the reporting state agency and data is transmitted to a CDC-Atlanta server. Upon completion of data entry and submission, a data message will be immediately sent to CDC-DVBID so that the personnel in the Arbovirus Disease Branch and WN Virus Surveillance Group may monitor reports of

WN virus infections in a continuous fashion. Also, after data entry and submission, these numerator data will become available on the CDC Secure Data Network so that authorized personnel from the reporting state agency may "approve" (proofread, and correct) individual numerator data records.

In the fourth method, the data transmitted via telephone, FAX, or e-mail will be entered into a database by CDC-DVBID personnel. Following data entry, DVBID personnel will return a short data message to the reporting state agency. This message will contain the data that were entered and allow the reporting state agency to immediately proofread and correct any errors. On a continuous basis, these DVBID-entered data will be transmitted to the CDC-Atlanta server where data will be available for review and approval on the CDC Secure Data Network. Following this approval, these data will be handled in the same manner as above.

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 approve over the CDC Secure Data Network. The UID will not appear in output products for public release. Most states already have systems in place for generating UIDs, and they should continue to use them. The CDC numerator databases will accommodate numeric or alphanumeric UIDs up to 25 characters long. States are encouraged to begin their UIDs with the 2-letter postal code for the state (or "NYC" for New York City).

The issue of numerator data records associated with laboratory-probable results deserves special mention. Although CDC strongly encourages attempts to confirm 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, in the midst of 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, *i.e.*, results for this bird will remain laboratory-probable (see table below). Furthermore, that state may decide to authorize DVBID staff to upload that bird's numerator data record to the CDC/Atlanta holding database, and subsequently authorize CDC to release it publicly. In contrast, a state may delay the release of such results to the public until they have been laboratory-confirmed. Therefore, CDC will rely on individual states to decide when to authorize the public release of numerator data records based on *laboratory-probable* results in mosquitoes, sentinel species, ill veterinary (non-avian) species, live, captured birds (*i.e.*, avian seroprevalence surveillance) and dead birds.

DVBID will not report numerator data records associated with laboratory-equivocal results, pending the results of further laboratory tests.

In terms of human surveillance, the national surveillance case definition of arboviral encephalitis in humans, adapted for use in WN encephalitis cases, includes two official case-status categories, *i.e.*, confirmed and probable (Table 3). For national arboviral encephalitis surveillance, CDC has traditionally reported these two categories together in its annual summary maps and other graphics, and will continue this practice within

the WN virus surveillance system. States are encouraged to promptly report to DVBID staff by telephone both laboratory-confirmed and laboratory-probable human WN encephalitis cases as numerator data records. In addition, states are encouraged to report "laboratory-equivocal" human cases in the same manner, *although DVBID staff will not upload such records to the CDC/Atlanta fileserver until and unless they are reclassified as laboratory-confirmed or -probable cases.*

Pending the appearance of surveillance case definitions for veterinary (non-avian) disease due to WN viral infection, the national surveillance case definition of arboviral encephalitis in humans should be used.

Arboviruses other than WN virus:

It is anticipated that enhanced surveillance for WN virus will result in increased recognition of infections with other arboviruses, including eastern equine encephalitis (EEE), western equine encephalitis (WEE), St. Louis encephalitis (SLE) and LaCrosse (LAC) viruses. Surveillance "numerator" data regarding these viruses may be reported to CDC-DVBID via telephone, FAX, or e-mail in the same manner as for WN virus. Web-based reporting of "numerator" data for these other viruses is not available.

Data Security Issues:

General principles:

- State and local health authorities will retain control of the timing of data release.
- CDC will provide data submitting authorities early and secured access to summary data from the surveillance system to ensure that error correction occurs before data are made available to the public, and to provide time to prepare for public data release.
- Personal identifying or localizing (more specific than county) information will not be released.
- Information of exceptional public health importance such as the identification of WN virus in a new area may require rapid release to the public health community. Such a release would occur only with the consent and collaboration of the authorities who reported the data to CDC.

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 Secure Data Network (SDN) which provides data encryption for transmission via the internet. To use SDN, users must obtain and install a digital certificate from the CDC certificate server. This allows for the identification of the computer/browser that is accessing a secure web site.

- To obtain a digital certificate and be approved to use the SDN, CDC's certificate authority at DVBID must approve the request and forward it to 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 denominator data to CDC, as well as those who will approve numerator and denominator data on the SDN.

Summary Reports to be Produced by CDC and the National Atlas/U. S. Geological Survey (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/National Atlas Project (a U. S. government-wide project directed by USGS). Maps and corresponding graphs and tables will be updated at least weekly on the National Atlas web site (www.nationalatlas.gov/federal.html).

Communication Issues:

- A dedicated telephone line (970-266-3592), electronic mailbox (dvbid2@cdc.gov), and fax machine (970-266-3599) is available at DVBID (in Fort Collins, Colorado) 24 hours/day for reporting numerator data or other urgent WN virus-related business. During nights and weekends, calls to the dedicated phone line will be forwarded to the cellular phone of an on-call 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 a weekly conference call between CDC, cooperating states and other federal agencies, Epi-X (or a similar secured communication network) that can provide an WN virus information exchange forum will be established.

  Epi-X is a password-protected internet portal that provides access to public health data and publications. It also provides a web-based, secured WV virus communication forum consisting of a variety of "conferences" on various WN virus-related topics. Some conferences will be accessible to all participants, while others will be restricted. Specific participation in the WN virus forum of Epi-X or a similar secured communication network must be approved by either a state epidemiologist or a state public health veterinarian. Federal employees must be approved by Dr. Duane Gubler of (CDC), Dr. Randy Crom (U. S. Department of Agriculture), or Dr. Bob McLean (USGS). For further information, contact the Arbovirus Disease Branch of DVBID at 970.221.6400 or send electronic mail to dvbid2@cdc.gov.

Submission of laboratory specimens to CDC for WN virus testing: See Table 5.

Table 1. "Denominator" Data Variable List

I. *Avian mortality:* Includes ill or dead birds that are not included in *sentinel species* or *seroprevalence surveillance* databases

- Year
- MMWR week that bird found ("MMWR week found")
  (Note: "MMWR week found" 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 found" should remain associated with this specimen **throughout testing.**)
- County
- State
- Number of reported crows by "MMWR week found" and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)
- Number of crows **tested** by "MMWR week found" and by county (Data source: Testing labs through state)
- Number of other reported birds by "MMWR week found" and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)
- Number of other birds **tested** by "MMWR week found" and by county (Data source: Testing labs through state)

(Note: "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.)

II. *Mosquito trapping:*
:

- 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: State, county or township WN virus surveillance coordinators through the state to CDC)
- Number of mosquitoes **tested** by MMWR week of collection, by county, and by species (Data source: Testing labs through state).

(Note: "Positive" results are reported through the "numerator" system by the testing facility/agency. In this report, the date of field collection is routinely obtained.)

III. *Sentinel species:* Includes sentinel groups (*e.g.*, flocks, herds) that are always in place and systematically and regularly sampled to detect seroconversion. Mammals or birds that are sampled on a one-time basis specifically to determine the seroprevalence of anti-WN virus antibody are included in *seroprevalence surveillance*

- Year
- MMWR week of serum collection
  (Note: This is the MMWR week that corresponds to the date of the serum collection. This date should remain associated with this serum specimen **throughout testing.**)
- County
- State
- Species (Horse, chicken, other)
- Number of individual animals bled by MMWR week of serum collection and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)
- Groups bled by MMWR week of serum collection and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)
- Groups in place by MMWR week of serum collection and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)

(Note: "Positive" results are reported through the "numerator" system by the testing facility/agency. In this report, the date of serum collection is routinely obtained.)

IV. *Seroprevalence surveillance:* Includes live, trapped *non-ill* mammals or birds species that are sampled specifically to determine the seroprevalence of anti-WN virus antibody. This ***excludes*** "sentinel groups" that are systematically/regularly sampled

- Year
- MMWR week of serum collection
  (Note: This is the MMWR week that corresponds to the date of the serum collection. This date should remain associated with this serum specimen **throughout testing.**)

- County
- State
- Species (equine, other mammal, crow, blue jay, pigeons, sparrows, other bird)
- Number bled by MMWR week of serum collection date and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)

(Note: "Positive" results are reported through the "numerator" system by the testing facility/agency. In this report, the date of serum collection is routinely obtained.)

V. *Veterinary:* Includes ill or dead non-human mammals that are not included in *sentinel species* or *seroprevalence surveillance* databases

- Year
- MMWR week of illness onset
  (Note: This is the MMWR week that corresponds to the illness onset date. This date should remain associated with this serum, CSF, or tissue specimen **throughout testing.**)
- County
- State
- Species (Equine, feline, canine, other)
- Number of non-human mammals from which specimens were **collected** for WN virus testing by MMWR week of illness onset and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)
- Number of non-human mammals for which specimens were **tested** for WN virus infection by MMWR week of illness onset and by county (Data source: Testing labs through state)

(Note: "Positive" results are reported through the "numerator" system by the testing facility/agency. In this report, the date of illness onset is routinely obtained.)

VI. *Human:* Includes ill or dead humans for whom a diagnosis of WN virus infection is being considered and for whom a clinical sample has been submitted

- Year
- MMWR week of illness onset
  (Note: This is the MMWR week that corresponds to the date of illness onset for the person. This date should remain associated with this clinical sample **throughout testing.**)
- County
- State
- Number of persons from whom specimens were **collected** by MMWR week of illness onset and by county (Data source: State, county or township WN virus surveillance coordinators through the state to CDC)
- Number of persons from whom specimens were **tested** by MMWR week of illness onset and by county (Data source: Testing labs through state)

(Note: "Positive" results are reported through the "numerator" system by the testing facility/agency. In this report, the date of illness onset is routinely obtained.)

Table 2. Numerator Data Variable List:

Surveillance Type	Basic numerator data collected by DVBID, for inclusion in a master database (for eventual public release)	Additional information to be collected by DVBID (for internal use only)	Additional information to be collected by DVBID only if laboratory testing at CDC is requested (for internal use only)
Mosquito	• state • county • pool UID • date of mosquito collection • week of collection • species	• mosquito pool information (species, sex, pool size) • available laboratory results (test type, results) • case status	• available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Sentinel species	• state • county • group UID • date of serum collection • week of collection • species	• group information (group size, evidence of illness in infected animals) • available laboratory results (test type, results) • case status	• available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Avian mortality	• state • county • bird UID • week bird found dead • species* • date bird reported or collected (earlier date) * Including “captive species”	• available laboratory test results (test type, results) • case status	• available necropsy results (list of specimens available) • available laboratory test results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Avian seroprevalence surveillance	• state • county • bird UID • week bird trapped & bled • species • date bird trapped	! available laboratory test results (test type, results) ! age (hatch-year, after hatch-year) ! trapping information (number of birds of these species trapped in this collection, evidence of illness in infected birds) ! case status	• available laboratory test results (list of specimens available) • sample disposition (arrangements for shipment of specimens)

Surveillance Type	Basic numerator data collected by DVBID, for inclusion in a master database (for eventual public release)	Additional information to be collected by DVBID (for internal use only)	Additional information to be collected by DVBID only if laboratory testing at CDC is requested (for internal use only)
Veterinary (non-avian)	• state • county • animal UID • week of illness onset • species (canine, equine, feline, or "other") • date of illness onset	• patient information (exact species, age, residence location below county level (*optional)*, clinical manifestations, fatal?, recent travel history), • available laboratory results (test type, results) • case status	• available necropsy results (list of specimens available) • available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Human	• state • county • patient UID • week of illness onset • date of illness onset	• contact information • patient information (age, sex, residence location below county level, date of onset, clinical manifestations, fatal?) • available autopsy results (facility, phone #, date of autopsy, results) • available laboratory results (facilities, phone #, specimen types, collection date, date tested, test type, results) • case status	• patient information (name, recent travel history, flavivirus vaccination history), • available autopsy results (list of specimens available) • available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)

Table 3. West Nile Virus Laboratory and Surveillance Case Criteria

Laboratory case definitions:

Surveillance Type	Laboratory-confirmed WN virus infection*	Laboratory-probable WN virus infection	Laboratory-equivocal WN virus infection
Mosquito	• WN virus isolation (identity of virus established by IFA using specific monoclonal antibodies, cross-neutralization, RT-PCR, or gene sequencing) • Positive RT-PCR test for WN viral RNA with validation by 1) repeated positive RT-PCR using different primers, 2) positive PCR result using another system (*e.g.*, TaqMan), or 3) virus isolation. • Capture of WN viral antigen validated by results of inhibition test	• Positive RT-PCR test for WN viral RNA in a single test	• Flavivirus isolation
Sentinel species	• WN virus isolation (identity of virus established by IFA using specific monoclonal antibodies, cross-neutralization, RT-PCR, or gene sequencing) • Seroconversion to WN virus in serially collected serum specimens, by plaque-reduction neutralization** • Detection of IgM antibody to WN virus, validated by demonstration of neutralizing antibody to WN virus**	• Detection of IgM antibody to WN virus	• Flavivirus isolation • Seroconversion to WN virus in serially collected serum specimens, by tests other than EIA or PRNT
Avian mortality	• WN virus isolation (identity of virus established by IFA using specific monoclonal antibodies, cross-neutralization, RT-PCR, or gene sequencing) • Positive RT-PCR test for WN viral RNA with validation by 1) repeated positive RT-PCR using different primers, 2) positive PCR result using another system (*e.g.*, TaqMan), or 3) virus isolation. • Detection of specific WN viral antigen in tissues (*e.g.*, by immunohistochemistry)	• Positive RT-PCR test for WN viral RNA in a single test • Detection of flaviviral antigen in tissues (*e.g.*, immunohistochemistry)	• Flavivirus isolation • Gross pathologic or histopathologic findings suggestive of WN viral infection
Avian seroprevalence surveillance	• Detection of neutralizing antibody to WN virus • Detection of antibody to WN virus, validated by demonstration of neutralizing antibody to WN virus**	• Detection of antibody to WN virus without detection of neutralizing antibody to WN virus	• Any serologically equivocal results (see below)

Surveillance Type	Laboratory-confirmed WN virus infection*	Laboratory-probable WN virus infection	Laboratory-equivocal WN virus infection
Veterinary (non-avian)	• As for humans (see below)	• As for humans (see below)	• Flavivirus isolation • Any serologically equivocal results (see below)
Human	• See below	• See below	• Flavivirus isolation • Any serologically equivocal results (see below)

* CDC strongly encourages attempts to confirm all laboratory-probable and -equivocal results.

** SLE virus infection should be ruled-out by cross-neutralization.

Surveillance case definitions:

Humans:

(Modified from: "CDC. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control"[26] at *www.cdc.gov/ncidod/dvbid/arbovirus_pubs.htm* and "CDC. Case definitions for infectious conditions under public health surveillance" at *MMWR* 1997;46:12-3 or http://www.cdc.gov/epo/dphsi/casedef/enceph97.htm)

The following working surveillance case definition of WN encephalitis was used in the 1999 New York epidemic and is an adaptation of the national arboviral encephalitis surveillance case definition.[26] public health tool intended only for the surveillance of health events in populations. It is neither 100% specific nor 100% sensitive, and it is not intended for use in clinical diagnosis or management decisions in individual cases. It should also be emphasized that the current national arboviral encephalitis surveillance case definition was approved and implemented by the Council of State and Territorial Epidemiologists – in consultation with CDC -- at a time when SLE virus was the only neurotropic flavivirus with epidemic potential known to occur in the U.S.[26] However, it is now conceivable that WN and SLE viruses coexist in this country. Antibodies to these closely related neurotropic flaviviruses and dengue viruses, which are increasingly imported, cross-react extensively in enzyme immunoassays (EIA) and hemagglutination-inhibition (HI) tests, to a lesser extent, in neutralization tests. (To an even lesser extent, serologic cross-reactivity also occurs between these two viruses and Powassan virus, a tick-borne flavivirus endemic to the northeastern U.S. and eastern Canada and which causes rare, sporadic, encephalitis cases in humans.) Thus, in future epidemics and sporadic viral encephalitis cases alike, the potential for initial misclassification of SLE cases as WN encephalitis cases -- and vice versa -- must be recognized and addressed, mainly by the use of cross-neutralization tests of serum or cerebrospinal fluid (CSF) or both, by virus isolation, or by detection of viral genome or antigens. Once WN virus (or SLE virus) has been determined to be the cause of an epidemic/epizootic (*e.g.*, by cross-neutralization tests and/or virus isolation from, or direct virus detection in, humans, birds, or mosquitoes), further cross-neutralization tests generally may be unnecessary to classify human cases for surveillance purposes. While theoretically possible, concurrent epidemics of SLE and WN encephalitis in the same area should be unlikely, particularly in temperate areas where the near-simultaneous introduction of both viruses would be required. In any case, epidemiologically, clinically, and in terms of prevention and control methods, the differences between these two viruses generally are subtle and largely academic.

Confirmed case: A confirmed case of WN encephalitis is defined as a febrile illness associated with neurologic manifestations ranging from headache to aseptic meningitis or encephalitis, plus at least one of the following:

- Isolation of WN virus from, or demonstration of WN viral antigen or genomic sequences in, tissue, blood, CSF, or other body fluid;[1]
- Demonstration of IgM antibody to WN virus in CSF by IgM-capture EIA;[2-4]
- A $\geq$4-fold serial change in plaque-reduction neutralizing (PRNT) antibody titer to WN virus in paired, appropriately timed serum or CSF samples;[2, 3, 5]
- Demonstration of both WN virus-specific IgM (by EIA) and IgG (screened by EIA or HI and confirmed by PRNT) antibody in a single serum specimen.[2, 4-6]

Probable case: A probable case is defined as a compatible illness (as above) that does not meet any of the above laboratory criteria, plus at least one of the following:

- Demonstration of serum IgM antibody against WN virus (by EIA);[3, 4]
- Demonstration of an elevated titer of WN virus-specific IgG antibody in convalescent-phase serum (screened by EIA or HI and confirmed by PRNT).[3-6]

Non-Case: A non-case is defined as an illness that does not meet any of the above laboratory criteria, plus:

- A negative test for IgM antibody to WN virus (by EIA) in serum or CSF collected 8-21 days after onset of illness;[3, 4]
  and/or
- A negative test for IgG antibody to WN virus (by EIA, HI, or PRNT) in serum collected $\geq$22 days after onset of illness.[3-5]

Notes:

1. Although tests of tissues or fluids by PCR, antigen detection, or virus isolation can be used to confirm WN encephalitis cases, they cannot be used to rule-out cases because the negative predictive values of these test methods in this disease are unknown.
2. See the above discussion concerning serologic cross-reactivity between WN and SLE viruses. Prior to a more definitive demonstration of WN virus as the cause of an epidemic or a sporadic viral encephalitis case, this serologic criterion should be used to classify human cases as *probable* only, pending definitive identification of the circulating flavivirus type (see discussion above).
3. Although the antibody response to human infection with WN virus has not been thoroughly or systematically studied, the following are reasonable assumptions, based on extensive experience with other flaviviruses, or preliminary conclusions based on empirical observations made during the 1999 and 2000 New York epidemic of WN encephalitis:
   - IgM antibody in serum: By the eighth day of illness, a large majority of infected persons will have detectable serum IgM antibody to WN virus; in most cases it will be detectable for at least 1-2 months after illness onset; in some cases it will reach undetectable levels prior to 1 month after illness onset; in some cases it will be detectable for 12 months or longer.
   - IgG antibody in serum: By 3 weeks post-infection (and often earlier), virtually all infected persons should demonstrate long-lived serum IgG antibody to WN virus by EIA, HI, and PRNT.
   - IgM antibody in CSF: In WN encephalitis cases, IgM antibody will virtually always be detectable in CSF by the eighth day of illness and sometimes as early as the day of onset; the duration of WN virus-specific IgM antibody in CSF has not been studied.
   - IgG antibody in CSF: IgG antibody in CSF often does not reach detectable levels and thus is a relatively insensitive indicator of infection.
   - Specificity of IgM-capture EIA: Serum (and CSF) from recently WN virus-infected persons will cross-react in IgM-capture EIAs when either WN virus or any closely related flavivirus is used as antigen. The homologous (infecting) serotype should be determined by cross-neutralization.
   - Specificity of IgG EIA: WN viral IgG antibody detectable by EIA (or HI) is broadly cross-reactive with all closely related flaviviruses, and this usually cannot be resolved with comparative EIAs (or HIs) using various flavivirus antigens. The homologous serotype should be determined by cross-neutralization.
   - Specificity of PRNT: In previously WN virus-infected persons *without* an antecedent history of infection with another flavivirus (*e.g.*, yellow fever vaccine virus or dengue), serum cross-neutralization tests against a battery of flaviviruses will usually implicate WN virus as the homologous virus. Serum from previously WN virus-infected persons *with* an

- antecedent history of infection with another flavivirus is often broadly cross-reactive by PRNT against a variety of other flaviviruses, and comparative titers are often insufficiently different to implicate the homologous virus.

Based on these assumptions or preliminary conclusions:

- Persons whose acute-phase serum or CSF specimens (collected 0-7 days after illness onset) test negative for IgM antibody to WN virus should have convalescent-phase serum specimens submitted for testing. Generally, convalescent-phase specimens should be drawn at least 2 weeks after acute-phase specimens. These intervals are arbitrary and not part of the national arboviral encephalitis surveillance case definition. In some cases, for example, seroconversion to WN virus can be demonstrated in specimens collected only a few days apart during the late acute or early convalescent phase of the illness.
- Negative tests for IgM antibody to WN virus in serum specimens collected more than 3 weeks after illness onset could be due to rapid waning of antibody; these results should be considered as potential false-negatives, pending IgG antibody testing.
- The EIA (or HI) for serum IgG antibody is a sensitive but relatively nonspecific test for previous WN virus infection. Positive results should be confirmed by PRNT.
- CSF should generally not be tested by WN viral IgG EIA (or HI). Instead, it should usually be reserved for testing by IgM-capture EIA and possibly by other means, including virus isolation, PCR, and neutralization.

4. At CDC, EIA results are based on "P/N ratios", which are optical density (OD) ratios or signal-to-noise ratios, not titers. A P/N ratio is calculated by dividing the OD of the test sample, P, by the OD of a normal, N, human antibody control. At CDC, serum specimens are routinely tested at a dilution of 1:400 and CSF specimens are tested undiluted. Empirically, CSF P/N ratios of ≥ 3 are considered positive for flavivirus IgM antibody at CDC, and serum IgM P/N ratios of 2.00-2.99 are considered to be equivocal pending further serologic testing (*e.g.*, EIA endpoint titration), and ratios <2 are is considered uninterpretable if the OD of the test sample with viral antigen is ≤ 2 times the OD of the test serum with normal mouse brain antigen. Because of the potential for interlaboratory variability in P/N ratios generated for identical serum samples, appropriate positive, negative, and equivocal ranges of P/N ratios must be empirically determined by each laboratory.
5. At CDC, a serum PRNT titer of 10 (*i.e.*, a 1:10 dilution of serum neutralizes at least 90% of the test virus dose) or greater is considered positive.
6. Longitudinal studies of WN encephalitis cases have shown that WN virus-specific IgM antibody can persist in serum for 12 months or longer. Thus, the presence of serum anti-WN viral IgM antibody is not necessarily diagnostic of *acute* WN viral infection. For this reason, especially in areas where WN virus is known to have circulated previously, suspected cases of acute WN encephalitis or meningitis should be confirmed by the demonstration of WN virus-specific IgM antibody in CSF, the development of WN virus-specific IgG antibody in convalescent-phase serum, or both.

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 take place over time, as dictated by feedback, experience, technical issues, and events.

A. National map: United States map with state boundaries; action buttons will allow the selection of each of the following categories (two maps or tables for each category, one reflecting the current week's data and the other reflecting cumulative data):
   1. Mosquito surveillance:
      a. Map showing each state as WN virus-positive, WN virus-negative, or blank (no data)
   2. Sentinel chicken surveillance:
      a. Map showing each state as WN virus-positive, WN virus-negative, or blank (no data)
   3. Avian morbidity/mortality surveillance:
      a. Map showing each state as WN virus-positive, WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset (cumulative national data)
   4. Veterinary (non-avian) surveillance:
      a. Map showing each state as WN virus-positive (# cases), WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset (cumulative national data)
   5. Human surveillance:
      a. Map showing each state as WN virus-positive (# cases), WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset (cumulative national data)

B. State Maps: Selecting an individual state from the national map will produce a map of that state with its county boundaries; action buttons will allow the selection of each of the following categories (two maps or tables for each category, one reflecting the current week's data and the other reflecting cumulative data):
   1. Mosquito surveillance:
      a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data)
   2. Sentinel species surveillance:
      a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data) by sentinel species (*e.g.*, horse, chicken)
   3. Avian mortality surveillance:
      a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset (cumulative state data)
   4. Avian seroprevalence surveillance:
      a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset (cumulative state data)
   5. Veterinary (non-avian) surveillance:
      a. Map showing each county as WN virus-positive (# cases), WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset, by species (cumulative state data)
   6. Human surveillance:
      a. Map showing each county as WN virus-positive (#cases), WN virus-negative, or blank (no data)
      b. Graph showing number of cases by week of onset (cumulative state data)

Table 5. Instructions for Submitting Laboratory Specimens to CDC for WN Virus 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 by made for CDC to conduct initial and/or confirmatory tests of tissues 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 specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of the state public health laboratory, National Veterinary Services Laboratory, USDA, or other 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.

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, 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.

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) Appendix D or comparable form. Form 5034 is available electronically at: *http://www.cdc.gov/ncidod/dvbid/arbovirus_pubs.htm. Some circulating versions of Form 5034 lack spaces for a patient's name. Nevertheless, please always include the patient's name when using any version of Form 5034 or other submission form.*

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.

CSF: Store in externally threaded plastic tubes. Ship at least 1.0 mL per specimen.

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

Human tissues: In suspected cases of arboviral encephalitis in which an autopsy is performed, both fresh-frozen and formalin-fixed 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).

Fresh-frozen material should be shipped on dry ice to CDC/Fort Collins at the above address.

After consulting with Dr. Sherif Zaki or other CDC/Atlanta pathology staff member (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 C – Surveillance Case Definition for West Nile Virus Infection in Equines

Confirmed Case

Compatible clinical signs[1] plus one or more of the following:

- isolation of West Nile (WN) virus from tissues[2] ;
- an associated 4-fold or greater change in plaque-reduction neutralization test (PRNT) antibody titer to WN virus in appropriately-timed [3], paired sera;
- detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or cerebrospinal fluid (CSF) and an elevated titer (1:10 or greater) to WN virus antibody by PRNT in serum;
- detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or CSF and a positive polymerase chain reaction (PCR) for WN virus genomic sequences in tissues[2];
- detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or CSF and a positive immunohistochemistry (IHC) for WN virus antigen in tissue;
- positive IHC for WN virus antigen in tissue and a positive PCR for WN virus genomic sequences in tissues[2].

Probable Case[4]

Compatible clinical signs[1] plus one of the following:

- detection of IgM antibody to WN virus by IgM-capture ELISA in serum or CSF, but no elevated titer (negative at 1:10) to WN virus antibody by PRNT in serum[5]
- no positive PCR for WN virus genomic sequences tissues[2], and no positive IHC for WN virus antigen in tissue;
- positive PCR for WN virus genomic sequences in tissues[2];
- positive IHC for WN virus antigen in tissue.

Notes:

1 Clinical signs must include ataxia (including stumbling, staggering, wobbly gait, or in coordination) or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, blindness, lip droop/paralysis, teeth grinding, acute death.

2 Preferred diagnostic tissues from equine are brain or spinal cord; although tissues may include blood or CSF, the only known reports of WN virus isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.

3 The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.

4 An equine classified as a probable case should, if possible, undergo further diagnostic testing to confirm or rule out WN virus as the cause of clinical illness.

5 A negative PRNT on serum collected 22 days or more after onset of clinical illness will reclassify this equine as a non-case.

Assumptions on which case definitions are based:

- IgM-capture ELISA testing may be slightly nonspecific; cross reactions to closely related flaviviruses (*e.g.*, SLE virus) may occur.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WN virus or a closely related flavivirus has occurred, very likely within the last three months.
- 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.
- Neutralizing antibody detected in serum by PRNT indicates past exposure to WN virus; equine exposed to WN virus in 1999 or 2000 may test positive for neutralizing antibody by PRNT in 2001.

Appendix D – CDC Data and Specimen Handling (D.A.S.H.) Form 50.34

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control and Prevention (CDC)
National Center for Infectious Diseases
Atlanta, Georgia 30333

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

STUDY ID: _____ STATE CONTACT PERSON & PHONE NO.: ( ) –

(for CDC Use Only)
CDC LAB. CODE | CDC NUMBER | DATE RECEIVED Mo. Da. Yr.

Justification must be completed by State health department laboratory before specimen can be accepted by CDC. Please check the first applicable statement and when appropriate complete the statement with the.*

1. Disease suspected to be of public health importance. Specimen is:
   (a) ☐ from an outbreak.
   (b) ☐ from uncommon or exotic disease.
   (c) ☐ an isolate that cannot be identified, is atypical, shows multiple antibiotic resistance, or from a normally sterile site(s)
   (d) ☐ from a disease for which reliable diagnostic reagents or expertise are unavailable in State.
2. ☐ Ongoing collaborative CDC/State project.
3. ☐ Confirmation of results requested for quality assurance.

*Prior arrangement for testing has been made. Please bring to the attention of:
(name) _____

Name, Address and Phone Number of Physician or Organization:

STATE HEALTH DEPT. NUMBER: _____ STATE LOC: _____

DATE SENT TO CDC: Mo. Da. Yr. _____ Completed by: _____

PATIENT IDENTIFICATION NUMBER: _____

Have specimens from this patient been submitted previously ☐ YES ☐ NO

BIRTHDATE OR AGE _____ Mo. Da. Yr. SEX: ☐ M ☐ F

RACE: White ☐ Black ☐ Asian or Pacific Islander ☐ American Indian or Alaska Native ☐ Not Specific ☐

ETHNICITY: Hispanic ☐ Non-Hispanic ☐ Not Specific ☐

CLINICAL DIAGNOSIS:

ASSOCIATED ILLNESS:

DATE OF ONSET: Mo. Da. Yr. FATAL? ☐ YES ☐ NO

LABORATORY EXAMINATION REQUESTED: ☐ **AN**timicrobial Susceptibility ☐ **ID**entification ☐ **IS**olation ☐ **HI**stology ☐ **OT**her (Specify) _____ ☐ **SE**rology (Specific Test) _____

CATEGORY OF AGENT SUSPECTED: ☐ **BA**cterial ☐ **VI**ral ☐ **FU**ngal ☐ **RI**ckettsial ☐ **PA**rasitic ☐ **OT**her (Specify) _____

SPECIFIC AGENT SUSPECTED: _____

OTHER ORGANISM(S) FOUND: _____

ISOLATION ☐ YES ☐ NO | **NO. TIMES ISOLATED:** _____

SPECIMEN SUBMITTED IS: ☐ **O**riginal **M**aterial ☐ **P**ure **I**solate ☐ **M**ixed **I**solate

DATE SPECIMEN TAKEN: Mo. Da. Yr.

ORIGIN: ☐ **SO**il ☐ **FO**od ☐ **AN**imal (Specify) _____ ☐ **HU**man ☐ **OT**her (Specify) _____

SOURCE OF SPECIMEN: ☐ **BL**ood ☐ **SE**rum ☐ **CSF** ☐ **ST**ool ☐ **SP**utum ☐ **UR**ine ☐ **GA**stric ☐ **HA**ir ☐ **SK**in ☐ **TH**roat
☐ **WO**und (Site) _____
☐ **EX**udate (Site) _____
☐ **TI**ssue (Specify) _____
☐ **OT**her (Specify) _____

SUBMITTED ON: ☐ **EG**g ☐ **TI**ssue Culture (Type) _____ ☐ **AN**imal (Specify) _____ ☐ **ME**dium (Specify) _____ ☐ **OT**her (Specify) _____

SERUM INFORMATION: Mo. Da. Yr. Mo. Da. Yr.
☐ **AC**ute _____ ☐ **S3** _____

IMMUNIZATIONS: Mo. Da. Yr.
_____
_____
_____

TREATMENT: Drugs Used: DATE BEGUN Mo. Da. Yr. DATE COMPLETED Mo. Da. Yr.
_____
_____
_____

CLINICAL TEST RESULTS:

Sputum and Histological Findings: _____

Blood Counts: _____ Stool/Urine Exams: _____

Type Skin Tests Performed: Mo. Da. Yr. Strength Pos. Neg.
_____ _____
_____ _____

SIGNS AND SYMPTOMS:
☐ **FE**ver
Maximum Temperature: ____
Duration: _____ Days
☐ **CH**ills

SKIN:
☐ **MA**culopapular
☐ **HE**morrhagic
☐ **VE**sicular
☐ **E**rythema **N**odosum
☐ **E**rythema **M**arginatum
☐ **OT**her _____

RESPIRATORY:
☐ **RH**initis
PUlmonary
☐ **PH**aryngitis
CAlcifications
☐ **O**titis **M**edia
PNeumonia (type)_____
☐ **OT**her _____

CARDIOVASCULAR:
☐ **MY**ocarditis
☐ **PE**ricarditis
☐ **EN**docarditis
☐ **OT**her _____

GASTROINTESTINAL:
☐ **DI**arrhea
☐ **BL**ood
☐ **MU**cous
☐ **CO**nstipation
☐ **AB**normal Pain
☐ **VO**miting
☐ **OT**her _____

CENTRAL NERVOUS SYSTEM:
☐ **HE**adache
MEningismus
☐ **MI**crocephalus
HYdrocephalus
☐ **SE**izures
CErebral Calcification
☐ **CH**orea
☐ **PA**ralysis
OTher _____

MISCELLANEOUS:
☐ **JA**undice
☐ **MY**algia
☐ **PL**eurodynia
☐ **CO**njunctivitis
☐ **CH**orioretinitis
☐ **SP**lenomegaly
☐ **HE**patomegaly
☐ **L**iver **A**bscess/cyst
☐ **LY**mphadenopathy
☐ **MU**cous Membrane Lesions
☐ **OT**her _____

STATE OF ILLNESS:
☐ **SY**mptomatic
☐ **AS**ymptomatic
☐ **SU**bacute
☐ **CH**ronic
☐ **DI**sseminated
☐ **LO**calized
☐ **EX**traintestinal
☐ **OT**her _____

EPIDEMIOLOGICAL DATA:
☐ **SI**ngle Case ☐ **SP**oradic ☐ **CO**ntact ☐ **EP**idemic ☐ **CA**rrier

Family Illness: _____

Community Illness: _____

Travel and Residence (Location): Mo. Da. Yr.
☐ **Foreign:** _____
☐ **USA:** _____

Animal Contacts (Species): _____

Arthropod Contacts: ☐ **NO**ne ☐ **EX**posure Only ☐ **BI**te

Type of Arthropod: _____

Suspected Source of Infection: _____

PREVIOUS LABORATORY RESULTS/OTHER CLINICAL INFORMATION:
(Information supplied should be related to this case and/or specimen(s) and relative to the test(s) requested.

The types of specimens usually sent to CDC laboratories are serum specimens, reference cultures, or clinical specimens. To assist State health department laboratories and others in obtaining the information on the request form that NCID requires, the following tabulation for each of the 3 types of specimens should serve as a guide.

SERUM SPECIMENS

Required	Useful
Laboratory exam requested	Clinical information
Specific agent suspected	Signs, symptoms, etc.
Serum information*	
Immunization*	
Treatment*	
Epidemiologic data*	
Previous lab results	

REFERENCE CULTURES

Required	Useful
Laboratory exam requested	Isolation attempted
Category of agent suspected	Date specimen taken
Specific agent suspected	Number times isolated
Kind of specimen	Other clinical information
Origin of specimen	Clinical test results
Source of specimen	Signs, symptoms, etc.
Submitted on what medium	Other organisms found**
Previous lab results	Epidemiologic data*
Biochemical reaction (can be attached on a separate sheet)	Treatment*

CLINICAL SPECIMENS

Required	Useful
Laboratory exam requested	Other clinical information
Category of agent suspected	Clinical test results
Specific agent suspected	Signs, symptoms, etc.
Specimen submitted is	
Date specimen taken	
Source of specimen	
Epidemiologic data*	
Previous lab results	

The Reference and Disease Surveillance Booklet should be consulted for special requirement.

**Exercise good judgement to determine the relevance of these items.* Paired sera are required for viral and bacterial disease serology, a single serum is required for mycotic and parasitic diseases and for syphilis serology (congenital syphilis excepted). In all instances the date(s) of collection of serum specimens must be provided. Immunization history is required when such information relates to the serology requested, i.e., required for polio, measles, etc., not required for histoplasmosis, echinococcosis, etc. Information on treatment, such as administration of immune serum or globulin, antibiotics, etc., is often of great benefit when doing serology or identifying reference cultures. As much relevant epidemiologic data as can be obtained should be provided. History of travel and animal or arthropod contacts are required for those RDS in which this kind of information is clearly necessary. If any required item of information is not available after efforts have been made to obtain it, please so indicate.

**Bacterial cultures representing growth of a single or a few colonies on the same primary isolation agar plates from which the principal pathogen has been isolated and identified should not be submitted for identification unless clinical findings or other justification support such submissions.

REFERENCES:

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