Centers for Disease Control and Prevention

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

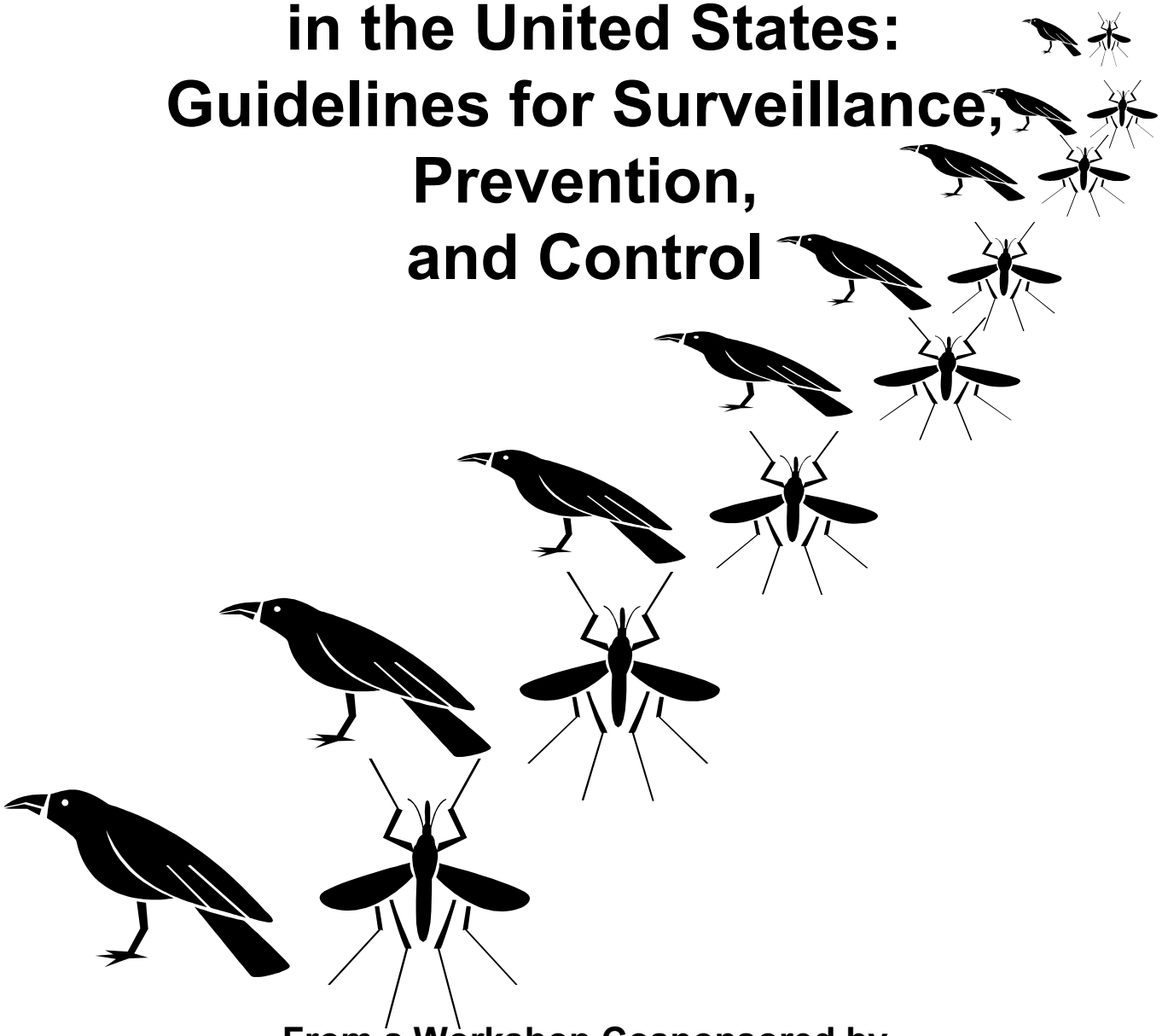

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Table of Contents

Executive Summary 1

Background/Introduction 4

I. SURVEILLANCE 5
- A. Ecologic Surveillance 6
  - 1. Avian 6
  - 2. Non-Human Mammals 10
  - 3. Mosquitoes 11
- B. Surveillance for Human Cases 12
  - 1. Clinical Syndromes to Monitor 12
  - 2. Types of Human Surveillance 12
  - 3. Specimens for Analysis 13
  - 4. Surveillance Case Definition 14
- C. Geography and Timing 14
  - 1. Northeastern United States 14
  - 2. Southern United States 14
  - 3. Western and Central United States 14
  - 4. Other Areas of the Western Hemisphere 15

II. LABORATORY DIAGNOSIS 15
- A. Serologic Laboratory Diagnosis 15
  - 1. Human 15
  - 2. Animal 16
- B. Virologic Laboratory Diagnosis 16
  - 1. Virus Isolation 16
  - 2. Virus Identification 17
- C. Biocontainment 17
  - 1. Laboratory Safety Issues 17
  - 2. Shipping of Agents 18
- D. Training and Infrastructure 18
  - 1. Front-Line Arbovirus Laboratories 18
  - 2. Training Programs 18
  - 3. Biocontainment 19

III. PREVENTION AND CONTROL 19
- A. Public Outreach 19
- B. Mosquito Abatement 19
- C. Protection of Domestic and Zoo Animals 19
- D. Legislative Issues 19

IV. HEALTH DEPARTMENT INFRASTRUCTURE 20
- A. Staffing and Personnel 20
- B. Training and Consultation 20
- C. Laboratory Capacity 20
  - 1. Testing for WN Virus Infections 20

V. INTERJURISDICTIONAL DATA SHARING AND NATIONAL REPORTING OF HUMAN CASES 21
A. Human Data 21
1. Clinical and Laboratory Data 21
2. National Reporting of Human Cases of WN Encephalitis 22
B. Ecologic Data 22
1. Accurate Taxonomic Identification of Specimens 23
2. Unique Identification (UID) Numbering System for Specimens 23
3. Durable Tagging System for Field-Collected Specimens 23
4. Standardized Data Collection and Specimen Submission Instruments . . . 23

VI. RESEARCH PRIORITIES 23
A. Current and Future Geographic Distribution of WN Virus 23
B. Bird Migration as a Mechanism of WN Virus Dispersal 24
C. Vector and Vertebrate Host Relationships and Range 24
D. Virus Persistence Mechanisms 24
E. Mosquito Biology, Behavior, Surveillance, and Control 24
F. Development and Evaluation of Prevention Strategies 24
G. Laboratory Diagnosis 24
H. Clinical Spectrum of Disease and Long-Term Prognosis in Humans 25
I. Risk Factor Studies 25
J. Viral Pathogenesis 25
K. Genetic Relationships and Molecular Basis of Virulence 25
L. Vaccine Development for Animals and Humans 25
M. Antiviral Therapy for WN and Other Flaviviruses 25
N. The Economic Cost of the Northeastern WN Virus Epidemic/Enzootic 25

Appendix A – Agenda and List of Meeting Participants 26

Appendix B – Questions/Issues Relating to West Nile Virus in the Western Hemisphere 38

Appendix C – Guidelines for Phased Response to West Nile Virus Surveillance Data 40

Appendix D -- Working Surveillance Case Definition of West Nile Encephalitis 41

Appendix E – CDC Data and Specimen Handling (D.A.S.H.) Form 50.34 44

REFERENCES 46

Executive Summary

The epidemic/epizootic of West Nile (WN) virus in the northeastern United States in the summer and fall of 1999 was an unprecedented event, underscoring the ease with which emerging infectious pathogens can move into new geographic areas. The outbreak also raised the issue of the preparedness of many local, state and national public health agencies to deal with epidemics of vector-borne diseases in this country. Because it is unknown whether WN virus will be able to persist through the winter, whether it has already or will spread to new geographic locations, and what the public health and animal health implications of this introduction will be, it is important to proactively establish surveillance, prevention and control programs to prevent future WN virus epidemics in this country. Accordingly, the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) co-sponsored a meeting of experts representing a wide range of disciplines (see Appendix A) to review the state of our knowledge about the epidemic/epizootic in the Northeast and to provide input and guidance on the kinds of programs that should be established to effectively monitor WN virus activity and to prevent potential future outbreaks of disease.

Surveillance

Enhanced surveillance was identified as a high priority for those states that were affected or that are at higher risk for being affected because of bird migration patterns. These include states from Massachusetts to Texas along the Atlantic and Gulf coasts, as well as countries in the Caribbean and Central and South America, underscoring the need for international cooperation. Depending on the geographic location of the state, active surveillance activities should be implemented now and continued through the winter months (southern states where mosquito activity is continuous throughout the year), or implemented early in the spring (northern states where mosquito activity has ceased because of cold weather). In all northeastern and southern states that face potential WN virus activity, the following surveillance activities should be emphasized:

1. Active bird surveillance. Monitoring of arbovirus activity in wild birds, sentinel birds, or both. Surveillance for dead crows, in particular, may be a sensitive means to detect the presence of WN virus in an area.

2. Active mosquito surveillance. Surveillance of mosquito populations to detect WN and other arbovirus virus activity, to help identify potential mosquito vectors in a particular area, and to monitor population densities of those vectors.

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.

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 cases of viral encephalitis and, if resources permit, aseptic meningitis.

Laboratory Diagnosis

Unequivocal diagnosis of WN virus or other arbovirus infections 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 will provide reagents and training as needed.

1. Serology. The immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assays (ELISA) should be available in all state public health and veterinary laboratories to provide the first-line testing for human and animal serum and cerebrospinal fluid specimens. In addition, selected state health and 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 laboratories and reference laboratories should have virus isolation and identification capabilities. These, plus selected other laboratories, should also have reverse transcriptase-polymerase chain reaction capability to detect viral RNA. All laboratory investigations that require handling live virus should be conducted under biosafety level 3 containment. Antigen-capture ELISAs should be developed to detect WN virus and other arboviruses in mosquito pools, and should be made available to state and local laboratories. 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.

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.

Research Priorities

Understanding how and why the 1999 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 identified at the workshop 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

Background/Introduction

The epidemic of West Nile (WN) viral encephalitis in the New York Metropolitan area in the summer of 1999 was unexpected and underscores once again the ease with which human and animal pathogens can move among the world's population centers. This epidemic also raised the issue of preparedness and the availability of appropriate public health infrastructure required to effectively monitor, prevent and control outbreaks of vector-borne diseases in the United States.

WN virus is a flavivirus belonging taxonomically to the Japanese encephalitis serocomplex that includes the closely related St. Louis encephalitis (SLE), Japanese encephalitis, Kunjin, and Murray Valley encephalitis viruses, as well as others.[1,2] WN virus was first isolated in the West Nile province of Uganda in 1937. The first recorded epidemics occurred in Israel during 1951-1954 and in 1957. European epidemics of WN encephalitis have occurred in southern France in 1962, in southeastern Romania in 1996, and in south-central Russia in 1999. The largest recorded epidemic caused by WN virus occurred in South Africa in 1974.

An outbreak of encephalitis in New York City and neighboring New York counties in late August and September 1999 was initially attributed to SLE virus, based on positive serologic findings in cerebrospinal fluid (CSF) and serum samples using a flavivirus-specific immunoglobulin M (IgM)-capture enzyme-linked immunosorbent assay (ELISA). The outbreak was subsequently shown to be caused by WN virus based on genomic sequencing of viruses detected in human, avian, and mosquito samples. The genomic sequences derived to date from human brain and from virus isolates from zoo birds, dead crows, horses, and mosquito pools are identical and most closely related to genomic sequences of WN virus strains from the Middle East.[3-5]

Although it is 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 New York, approximately 40% of laboratory-positive cases had severe muscle weakness; of these 20% developed flaccid paralysis with electromyographic findings consistent with axonal neuropathy.

Within its normal geographic distribution of Africa, the Middle East, western Asia, and Europe, WN virus has not been documented to cause natural epizootics in birds. Crows and other birds with antibodies to WN virus are common, suggesting that asymptomatic or mild infection usually occurs among birds in those regions, although one experimental study showed high mortality in hooded crows and house sparrows in Egypt.[6] Similarly, substantial virulence of SLE virus in birds has not been reported. Therefore, an epizootic producing high mortality in crows and other bird species is unusual for either WN or SLE viruses. Migratory birds may play an important role in the natural transmission cycles and dispersal of both viruses. Like SLE virus, WN virus is transmitted principally by *Culex* species mosquitoes, but it also has been isolated from *Aedes*, *Anopheles*, and other species. The predominance of urban *Culex pipiens* mosquitoes trapped during this outbreak, and the high WN virus infection rate in this species in New York, suggests an important role for this species. Surveillance for early detection of virus activity in birds and mosquitoes will be critical indicators for initiation of control measures.

The public and animal health implications of the introduction of WN virus into the Western Hemisphere are not known. Nor is it known how widely the virus has spread geographically or what kind of host-vector relationships will develop if it does spread to new areas. In an attempt to answer some of these questions, the Centers for Disease Control and Prevention (CDC) and United States 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. The meeting was held in Fort Collins, Colorado, on November 8-9, 1999.

The participants met in plenary session to review all available information on the 1999 epidemic/epizootic, followed by three discussion groups on surveillance, laboratory diagnosis, and vector control issues. A copy of the agenda and the participant list are attached to this report as Appendix A. All discussion groups were provided a series of questions (Appendix B) to help guide their discussions on requirements for monitoring and mitigating the future impact of WN virus on public and animal health.

Workshop participants agreed that the 1999 WN virus epidemic/epizootic in the Northeast was yet another wake-up call that public health officials must anticipate and be better prepared to respond to such surprises, and that the outbreak re-enforced the need for a better public health infrastructure at the local, state, and national level to control vector-borne diseases. Today's rapid transport of people, animals, and commodities makes it likely that other introductions of exotic pathogens will occur. There was general agreement that CDC should move as quickly as possible to fully implement the plan entitled "Preventing Emerging Infectious Diseases, a Plan for the 21st Century."[7] Moreover, there was agreement that CDC must assume the leadership role in developing a national response to the introduction of WN virus into the Western Hemisphere.

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 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, specifically those 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.

To detect without delay whether WN virus was able to persist through the winter months in the United States, intensified active surveillance must be developed and implemented in early 2000 in those areas along the Atlantic and Gulf coasts where, based on bird migration patterns, transmission is most likely to occur (catchment area). In northern states where mosquito activity ceased because of cold weather, active surveillance should be initiated in the early spring. An exception is the New York City areas where transmission was most intense in 1999; overwintering mosquitoes should be monitored for WN virus infection, and

control implemented in overwintering sites if feasible. In southern states where mosquito activity is continuous throughout the year, active surveillance should be maintained year-round.

Appropriate 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 virus activity is detected in the bird or mosquito surveillance systems (see Appendix C). A basic reference on arbovirus surveillance is: CDC Guidelines for Arbovirus Surveillance Programs in the United States.[8] 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

To detect future WN virus activity, regional surveillance programs that are flexible enough to be readily adapted to other vector-arbovirus systems should be maintained or established. Note: Standard biosafety precautions, including the use of protective gloves and clothing, should be taken when handling wild or domestic animals.[9] Ecologic surveillance may include the following:

1. Avian

a. Sentinel birds

Although an ideal 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 viremias sufficient to infect vector mosquitoes.[8] Nevertheless, sentinel birds should be one of the mainstays of ecologic surveillance programs for WN virus and other domestic arboviruses. Domestic chickens, for example, have been used extensively and effectively as sentinels in many surveillance programs for SLE, EEE, and WEE viruses in the United States, and for WN virus and closely related flaviviruses in Africa and Australia. Sentinel birds have never been shown to pose an increased risk of arbovirus infection to their handlers or the human population at large. Monitoring of seroconversion in farm and yard chickens can be used in addition to, or as an alternative to, sentinel chicken-based arbovirus surveillance programs.

Chickens are currently being evaluated as sentinels for WN virus. At CDC, preliminary laboratory studies of small numbers of chickens of various ages have shown that: 1) 100% of chickens were susceptible to WN virus infection by needle inoculation or when fed upon by infected mosquitoes, 2) chicken survival was 100% and WN virus-specific antibodies were detectable by 7 days post-infection by both antibody-capture EIA and neutralization, 3) WN virus was usually detectable in cloacal swabs, and 4) most chickens demonstrated 1-2 days of viremia at titers sufficient to infect at least some mosquitoes. Thus, based on these preliminary findings, chickens appear to meet two of four criteria for an ideal WN virus sentinel. However, the benefits of their use appear to

outweigh the associated risks. Moreover, a more ideal sentinel for WN virus has yet to be identified. Any theoretical risk posed by cloacal shedding of WN virus by sentinel chickens should be minimized by using standard biosafety precautions, including the use of protective gloves and clothing and proper disposal of wastes.[9] Because the biomass of sentinel chickens is very small when compared to that of the local wild bird population in any given area, any theoretical contribution of infected sentinel chickens to WN virus amplification in that area should be small and transient. This theoretical risk could be further reduced by the placement of flocks away from densely populated areas, the use of cages designed to trap mosquitoes that enter them, or removal of a given sentinel chicken flock once serconversion to WN virus has been detected. The removal of a seropositive sentinel flock should be accompanied by enhanced mosquito-based surveillance for WN virus in that area.

(1) Specimens

Whole blood can be collected in microtainers and centrifuged for serum.

(2) Advantages of sentinel surveillance

- There is a long history (> 6 decades) of successful use in flavivirus surveillance (chickens);
- Chickens, geese, and pigeons sampled in the Queens Borough of New York City, the epicenter of the recent epidemic, all had a high seroprevalence (e.g., >50% prevalence of neutralizing antibody to WN virus);
- These species are readily fed upon by *Cx. pipiens*;
- These species, which are adapted to captivity, can be serially bled, and the geographic location of infection is not in question;
- These species are relatively easy to bleed;
- Collection and handling of specimens (serum) are inexpensive;
- No necropsies are needed;
- The system is flexible and therefore can be expanded and contracted as appropriate;
- Mosquito-abatement districts can agree to maintain flocks, bleed birds, and submit specimens for testing;
- Laboratory expenses can be defrayed by charging nominal fees per test.

(3) Disadvantages of sentinel surveillance

- Sentinel flocks detect only focal transmission, requiring that multiple flocks be positioned in representative geographic areas;
- Flocks are subject to vandalism and theft, limiting their usefulness in urban areas;
- Set-up and flock maintenance are expensive (i.e., birds, cages, feed, transportation);
- A high proportion of standing chicken, goose, or pigeon flocks in some areas such as Queens, the epicenter of the 1999 epidemic, may already be seropositive to WN virus.

b. Wild crow surveillance

Crow-based surveillance should include at least two basic elements: 1) the timely reporting of die-offs by wildlife field staff and biologists and by the general public and 2) submission of selected individual birds for WN virus testing. Birds submitted for testing should be recently dead ($\leq$24 hours).

(1) Specimens

Necropsy tissues (brain, spleen and other tissues) for gross pathology, histopathology, polymerase chain reaction (PCR) testing, virus isolation and antigen detection. Ideally, only specimens with histopathologic evidence of WN virus should be forwarded.

(2) Advantages of crow surveillance

- American crows may be very susceptible to clinical disease from WN virus infection and thus experience a high clinical attack rate;
- Their size and coloration make them conspicuous;
- They occur in a wide variety of habitats ranging from urban to wilderness, and occur in large numbers in nature;
- Wildlife workers and bird enthusiasts are primed to detect crow (and other avian species) die-offs in the eastern United States;
- Reverse transcriptase-PCR can be used to rapidly detect WN viral RNA in tissues, even in some grossly necrotic specimens;
- Set-up or maintenance costs may be minimal.

(3) Disadvantages of crow surveillance

- Crow dispersal makes it difficult to know where the dead/dying crow acquired infection with WN virus;
- Collection, handling, shipping, and processing of birds or clinical specimens are cumbersome;
- Systems for handling, processing, and testing could be overwhelmed by excessive public response.
- The long-term usefulness of this system is uncertain since natural selection for disease-resistant crows may occur or the virus may change, resulting in low or no mortality.

c. Other avian species (e.g., passerines, ardeid birds, pigeons, Canada geese) surveillance

In each geographic area, the optimal species for serologic surveillance purposes should be determined by serosurveys. The best sentinels for serologic surveillance are those species in which infection is rarely if ever fatal. Avian serology (other than hemagglutination-inhibition and neutralization tests) requires a bank of species-specific antiserum. The responsibility for developing and distributing such a serobank will be shared by CDC, USDA and the U.S. Geological Survey (USGS).

(1) Specimens

Serum can be tested for antibody. Necropsy tissues from sick/dead birds can be studied by gross and histopathology, and tested by RT-PCR, virus isolation, and immunohistochemistry.

(2) Advantages

- Long history of successful use in flavivirus surveillance;
- Local movement of resident wild birds may allow contact with enzootic transmission foci, thus increasing sensitivity;
- Set-up or maintenance costs may be minimal.

(3) Disadvantages

- Movement of free-ranging wild birds makes it impossible to know where the infection was acquired;
- Free-ranging birds must be live-trapped for serum collection and permits are required;

- Bird capture and banding permits require careful determination of species identity, sex, and age;
- Venipuncture of small wild birds is technically difficult;
- It is generally not feasible to serially bleed individual free-ranging birds because of low recapture rates (although banding can be useful);
- Serologic testing may require species-specific antiserum;
- If local laboratories begin conducting avian serology, large volumes of reagents will be required and there may be quality control/assurance problems.

2. Non-Human Mammals

a. Horses

Veterinarians and veterinary service societies/agencies are essential partners in any surveillance activities involving horses with neurologic disease.

(1) Specimens

Serum and CSF for antibody testing; necropsy tissues for gross pathology, histopathology, PCR, virus isolation, and immunohistochemistry.

(2) Advantages

- Horses are highly conspicuous, numerous, and widely distributed in some areas;
- Some are routinely bled and tested for other pathogens.

(3) Disadvantages

- Horses are usually not a good “early warning” sentinel (e.g., human cases of EEE may occur simultaneously with or soon after horse cases);
- Necropsies are expensive and logistically difficult;
- Horses are not present or abundant in many areas of the United States.

b. Carnivores

It is not known whether WN virus-associated disease occurs in dogs and cats. There was a single, fatal WN virus infection occurred in a cat during the 1999 epidemic. The seroprevalence of WN virus among dogs and cats sampled in New York City was low.

(1) Specimens

Serum for antibody testing and brain tissue from fatal cases.

(2) Advantages

- In some states, brain specimens that test negative in rabies surveillance programs are readily available and can be used for WN virus surveillance;
- Samples from pet dogs and cats with neurologic disease can be taken during clinic visits.

(3) Disadvantages

- The usefulness of such samples for detecting flavivirus activity is unclear;
- Carnivores may be insensitive sentinels.

3. Mosquitoes

Mosquito surveillance, along with bird-based surveillance, should be the mainstay of most regional surveillance programs for arboviruses, including WN virus. Adult mosquitoes can be collected by using a number of traps, sorted by species, pooled, and tested for virus infection. Bloodmeal identification can be conducted to determine principal vertebrate hosts of mosquito species. During the winter of 1999-2000, hibernating adult *Culex* mosquitoes will be collected from the 1999 epidemic area and tested for WN virus. During the spring of 2000, CDC and others will collect newly emergent *Culex* mosquitoes from the 1999 epidemic area and test them for overwintering WN virus.

a. Specimens

Adult or larval mosquitoes for species identification and for virus detection.

b. Advantages

- May provide the earliest and most definitive evidence of transmission in an area;
- Provides information on potential mosquito vector species;
- Provides an estimate of vector species abundance;
- Provides information on virus infection rates in different mosquito species;
- Provides information on relative risk to humans and animals;

- Provides baseline data that can be used to guide emergency control operations;

- Allows evaluation of control methods.

b. Disadvantages

- Labor-intensive and expensive;

- Substantial expertise is required for collecting, handling, sorting, species identification, processing, and testing.

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 possibly 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.

1. 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.

2. Types of Human Surveillance

a. Enhanced passive surveillance

In the absence of known WN virus activity in an area, enhanced passive surveillance (i.e., 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) for hospitalized cases of viral 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

*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. Symptoms may include headache, stiff neck, confusion or other mental status changes, nausea, or vomiting. Signs may include fever, 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 New York City in 1999, approximately 40% of laboratory positive cases had severe muscle weakness; of these, 20% 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.

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 insure accurate interpretation of serologic results.

b. Active human surveillance

Active surveillance should be strongly considered in areas with known or anticipated 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 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. In addition, hospital discharge data could be monitored for an increase in hospitalizations for central nervous system infections, although the timeliness and utility of this approach have not been tested.

c. Syndromic surveillance

In some urban areas, syndromic surveillance systems are in place or being developed to detect potential bioterrorism events. The “piggy-backing” of surveillance for WN meningoencephalitis and milder clinical forms of WN fever, e.g., fever with rash or lymphadenopathy, onto existing systems, including those involving large health maintenance organizations, should be encouraged.

d. 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).

3. Specimens for Analysis

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 may also be isolated, or detected by RT-PCR, in acute-phase CSF samples.

b. Serum

Paired acute-phase (collected as early as possible after onset of illness) and convalescent-phase (collected ≥8 days after clinical onset) serum specimens are useful for demonstration of seroconversion to WN and other arboviruses by ELISA or neutralization test. 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 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 should be submitted to CDC or other specialized laboratories for arbovirus 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[10] (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).

C. Geography and Timing

1. Northeastern United States

Active ecologic surveillance and enhanced passive surveillance for human cases should begin in the early spring and continue through the fall of 2000 until mosquito activity ceases because of cold weather. Surveillance in urban and surrounding areas should be emphasized.

2. Southern United States

Because mosquito activity continues year-round and WN virus could conceivably circulate in some areas in winter, especially the Gulf States, active ecologic surveillance and enhanced passive surveillance for human cases should be initiated in the fall of 1999 and continued at least through the fall of 2000.

3. Western and Central United States

If WN virus is introduced to Central and South America by migratory birds, the possibility exists that the virus could be introduced to the western and central United

States. Increased awareness and enhanced surveillance should be initiated in the early spring of 2000.

4. Other Areas of the Western Hemisphere

   Development of surveillance systems capable of detecting WN virus activity should be encouraged in 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 periurban 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. Ultimately, a plan to develop laboratory capacity will be drafted in collaboration with the American Public Health Laboratories Association. 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 D.

A. Serologic Laboratory Diagnosis

Accurate interpretation of serologic findings requires knowledge of the specimen. 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) onset date; 2) date of sample collection; 3) unusual immunological status of patient (e.g., immunosuppression); 4) address and travel history, especially to flavivirus-endemic areas; 5) history of prior vaccination with a flavivirus (e.g., yellow fever, Japanese encephalitis, or Central European encephalitis); and 6) brief clinical summary with 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 ELISAs should be the front-line tests for serum.

   b. The WN virus strain Eg101 is currently the prototype WN virus used to prepare antigen for diagnostic testing, and it performed well during the 1999 outbreak. Comparisons of strain Eg101 to the strains of WN virus isolated in New York in

1999 should be undertaken to determine whether the New York strains should be substituted for antigen production.

c. To maintain Clinical Laboratory Improvements Amendments (CLIA) certification, CLIA recommendations for positive and negative ranges should be followed, and proficiency testing programs should be initiated by reference laboratories.

d. Since the ELISA is cross-reactive between SLE, dengue, yellow fever, Powassan, and WN viruses, it should be viewed as a screening test only. Initial serologically positive cases should be confirmed by neutralization test. After an outbreak has been confirmed as being caused by a single agent, the ELISA can generally be used to diagnose most subsequent cases.

2. Animal

a. In general, the procedures for animal serology should follow those used with humans cited above.

b. Plaque-reduction neutralizing test (PRNT) and hemagglutination-inhibition assays for various animal species, while technically more demanding, may be useful because they are species independent. However, PRNT requires live virus, precluding laboratories without BSL3 containment from performing this test.

c. Because of the need for anti-species ELISAs, USDA, USGS, and CDC will pursue the development of anti-species antibodies that can be used as ELISA detector antibodies.

B. Virologic Laboratory Diagnosis

While the systems in place during the 1999 WN virus outbreak generally functioned well, a number of animal pathogenesis issues remain to be answered that bear directly on the virology of WN virus infection. Notwithstanding these important issues, recommendations can be made with regard to virus isolation and identification procedures.

1. Virus Isolation

a. Virus isolation should only be attempted by reference, regional, or other laboratories that have certified BSL3 containment.

b. Virus isolation attempts should be performed in a variety of substrates, including neonatal mouse inoculation and cell cultures from both vertebrate and mosquito origin; mosquito cells may not show cytopathic effects and should be screened by immunofluorescence.

c. Appropriate samples for virus isolation include brain tissue, CSF and serum from humans, various organs and blood products from birds and other vertebrates, and mosquitoes.

2. Virus Identification

    a. Immunohistochemistry (IHC) on brain tissue has been very useful in identifying both human and animal 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.

    b. Antigenic analysis

        (1) An indirect immunofluorescence assay using well-defined murine monoclonal antibodies (MAbs) is the most efficient, economical, and rapid method to identify isolated 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.

        (2) A well-characterized antigen-capture ELISA is available for detection of SLE virus antigen. CDC will attempt to derive a similar assay for WN virus antigen.

        (3) Virus neutralization assays can also be used to differentiate viruses, by using four-fold or greater titer differences as the diagnostic criteria.

    c. Nucleic Acid Analysis

        (1) RT-PCR of tissues, mosquito pools, CSF, and serum has proven to be a reliable method for use in mosquito, avian, and human surveillance. Standardized protocols should be developed and disseminated by reference laboratories. Primer design information should be included so that other laboratories can prepare primers. A proficiency testing program should be developed by the reference laboratories so that these tests can be CLIA-certified in local laboratories.

        (2) Real-time PCR (TaqMan) should be developed to rapidly rule out other viral pathogens for which antivirals are available. CDC and the University of California-Irvine will pursue development of sensitive and specific TaqMan assays for virus identification from human, animal, and mosquito specimens.

C. 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. As such, laboratory investigations that involve handling of

virus requires BSL3 containment. Specifications for BSL3 containment are available.[10] Concerns were 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. Because of this, a number of recommendations were provided:

(1) Since WN virus may be present in acute-phase serum and CSF specimens, aliquots to be used for serology should be heat-inactivated at 56°C for 30 min if testing is to be performed by laboratories with only BSL2 containment. The rest of the sample should be stored at -70°C without heat-inactivation.

(2) Handling of clinical material under BSL2 containment should proceed in Class 2 biological safety cabinets which are located in laboratory rooms with restricted access.

(3) Aerosol-producing procedures (e.g., ELISA plate rinsing) should be performed in a Class 2 biological safety cabinet.

(4) Animal studies should be conducted under BSL3 containment in USDA-approved facilities. A protocol for necropsy of horses can be obtained from USDA.

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

c. These biosafety recommendations should be reviewed and approved by both the SALS and CDC.

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 now required for transport of known WN virus isolates.

D. Training and Infrastructure

1. Front-Line Arbovirus Laboratories

Greater numbers of capable front-line laboratories performing screening assays (such as ELISA) should be developed to reduce time demands on reference laboratories.

2. Training Programs

Laboratory training programs should be developed at the regional and/or federal levels.

3. Biocontainment

Appropriate laboratories should have containment upgraded to BSL3.

III. PREVENTION AND CONTROL

Effective prevention and control of arbovirus diseases such as that caused by WN virus can only be accomplished by mosquito control and by preventive measures taken by the public to decrease the risk of mosquito bites.

A. Public Outreach

It is essential to educate the public about how WN virus and other arboviruses are transmitted, and about measures available to reduce exposure to mosquitoes that transmit these viruses. Public education and awareness of mosquito biology, behavior, and control issues should be enhanced by using social marketing methods. The New York City model--which used multi-lingual education materials on personal protective measures, including effective use of repellants, how to eliminate and control mosquito breeding sites in residential areas, and contingency plans for establishing a public hotline--can be used to help develop these programs.

B. Mosquito Abatement

Cost-effective mosquito control can only be accomplished through source reduction, i.e., control of larval mosquitoes before they emerge as adults. This requires an understanding of the species occurring in an area, accurate mapping of larval habitats, seasonal distribution, and behavior. The rationale is to monitor mosquito populations and implement control measures prior to the occurrence of human infections. The most cost-effective way to achieve this type of mosquito surveillance and control is through local mosquito abatement programs. These programs use professional vector biologists to develop the program using local resources, which usually requires authorizing legislation. There are several programs that can be used as models, including programs in California; Harris County, Texas; New Orleans, Louisiana; and Lee County and other Florida counties. These mosquito control programs all use Integrated Pest Management techniques and rely on surveillance information to direct control efforts. Baseline patterns of insecticide susceptibility should be evaluated.

C. Protection of Domestic and Zoo Animals

Susceptible domestic animals and zoo animals should be protected from mosquito contact. Mosquito source reduction is recommended for zoos and adjacent areas, and for farms.

D. Legislative Issues

States should be encouraged to develop specific legislation to authorize locally funded mosquito abatement programs.

IV. HEALTH DEPARTMENT INFRASTRUCTURE

Every state health department should have a functional arbovirus surveillance and response unit, staffed by well-trained personnel who have adequate data-processing resources, suitable laboratory facilities, and an adequate operating budget. The size and complexity of these units will vary by jurisdiction, depending on 1) the importance of arboviral diseases in the area and 2) available resources. A functional arbovirus surveillance unit should be considered an essential component of any emerging infectious diseases program.

A. Staffing and Personnel

Ideally, arboviral surveillance involves epidemiologists, virologists, medical entomologists, vertebrate biologists, veterinarians, 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.

B. Training and Consultation

CDC should increase opportunities 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 United States 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 epidemic. 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 submitted to CDC or another laboratory capable of confirmatory 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.

V. INTERJURISDICTIONAL DATA SHARING AND NATIONAL REPORTING OF HUMAN CASES

The public and animal health response to the recent epidemic/epizootic of WN virus in the Northeast involved many levels of government primarily in the states of New York, Connecticut, and New Jersey, as well as the federal governments of the United States and Canada. Often, multiple agencies within each jurisdiction and governmental level were involved. The need for more rapid, efficient, secure, and better coordinated ways of sharing both human and ecologic data between different agencies during such a crisis was underscored. A distinction was made between those systems needed to support an epidemic response and those needed to support long-term surveillance activities, although some overlap exists.

During an epidemic involving multiple jursidictions, CDC should take the lead in rapidly making available a system of electronic communication, e.g., list servers and web sites, to facilitate the rapid, efficient, and secure exchange of information between authorized users. Such a system should be integrated with CDC's other systems and not be a stand-alone system, which is incompatible with existing and planned systems. Tools currently being developed for response to bioterrorism events should be adapted for general use in multijurisdictional epidemic response. 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, while others do not; the converse is also true. Geographic information system (GIS) should be used to track epidemics more accurately.

A. Human Data

1. Clinical and Laboratory Data

CDC should take the lead in developing generic templates for electronic databases that can rapidly be customized and stored centrally to allow efficient and secure interjurisdictional sharing of human clinical and laboratory data during epidemics. Issues include:

a. 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.

b. 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, and the like, to limit access and minimize opportunities for breaches in confidentiality or security.

c. 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. At least temporarily, while more specific instruments are rapidly developed and disseminated, standard form CDC 50.34 ("the D.A.S.H. form") can be used as a generic instrument for the collection of clinical and epidemiologic data (available at web site www.cdc.gov/ncidod/dvbid/pubs.htm and as Appendix E). A completed CDC 50.34 is also a standard requirement when submitting clinical samples to CDC laboratories for testing. The characteristics of data variables (i.e., types, names, lengths, and order) contained in centralized electronic databases that are developed should follow guidelines or standards currently being developed by CDC and its partners for general use in public health data collection.

d. Centralization

During an epidemic, centralized electronic databases for sharing patient and laboratory information should be maintained at CDC or another central location where they can be accessed by authorized users via the Internet.

2. National Reporting of Human Cases of WN Encephalitis

Because the recent cases in New York are the first ever diagnosed in the United States, 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.[9] If future studies demonstrate the persistence of WN virus in the United States, CDC should propose to CSTE the addition of WN encephalitis to the nationally notifiable diseases list.

B. 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 mosquito vectors.

2. Unique Identification (UI) Numbering System for Specimens

   With CDC coordination, a standardized UI numbering system should be developed (or adopted from an existing system) for wide-scale use by all participants in a given catchment area. The numbering system should readily distinguish between each major animal group (i.e., humans, birds, and mosquitoes).

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 case investigation forms should be developed and used for birds, mosquitoes, and other animals. Some instruments already exist for internal or external use (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 public health and animal health implications of the introduction of WN virus to the United States and to the Western Hemisphere are unknown at this time. Many questions remain (see Appendix B), the answers to which will require considerable research. Workshop participants agreed unanimously that a research agenda be developed, with priority given to research questions whose answers can be directly applied to prevention and control. These research priorities are outlined below.

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). This must be a priority in the spring and summer of 2000.

B. Bird Migration as a Mechanism of WN Virus Dispersal

Experience in Europe and the Middle East suggests that WN virus is regularly introduced to new geographic areas along bird migration routes.[1,2] A better understanding of this potential is required for the Western Hemisphere.

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 United States 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 United States. 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, 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 research on new and innovative control methods for the mosquito vectors. Ultimately, prevention strategies must be integrated and use a variety of approachs to control mosquitoes as well as reduce the risk of transmission.

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 SLE, dengue, and yellow fever viruses. Tests for specific IgM 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 presentation 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 flaviriuses, 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 New York City and adjacent areas. These data will help set priorities for capacity building and prevention programs.

Appendix A – Agenda and List of Meeting Participants

Workshop on West Nile Virus in the U.S.
Agenda
November 8-9, 1999

November 8

0800-0805	Welcome	Dr. R. Curnow
0805-0810	Welcome	Dr. J. Hughes
0810-0815	Welcome	Dr. A. Torres
0815-0830	Introduction and charge to participants	Dr. D. Gubler
0830-0900	Epidemiology and clinical aspects of the NY WNV epidemic	Dr. M. Layton
0900-0930	Emergency mosquito surveillance and control	Dr. D. White/Dr. R. Nasci
0930-1000	Laboratory diagnosis/virus characterization	Dr. J. Roehrig/Dr. R. Lanciotti
1000-1030	Methods for Pathogen Discovery	Dr. I. Lipkin
1030-1045	BREAK	
1045-1115	Epizootic WNV Disease in Birds	Dr. N. Komar/Dr. R. McLean
1115-1145	WNV Infection in Horses	Dr. A Torres
1145-1215	Pathogenesis of WNV for humans, birds, and horses	Dr. S. Zaki/Dr. T. McNamara/Dr. W. Stone
1215-1315	LUNCH	
1315-1345	Overview of bird migration patterns	Dr. J. Rappole
1345-1415	Epidemic WNV in Volgograd, Russia	Dr. A. Platonov
1415-1500	Discussion	Group
1500-1730	Breakout Sessions	

EPIDEMIOLOGY — Long's Peak Room A201 (main conference room)
Moderator: Dr. D. Morse
Rapporteurs: Dr. J. Hadler, Dr. R. Campbell
Issues to Discuss:
Surveillance
- Human
- Animal

Health Department Infrastructure
Research Priorities

LABORATORY DIAGNOSIS/VIROLOGY — Buffalo Peak A220 (Director's conference room)
Moderator: Dr. J. Mackenzie
Rapporteurs: Dr. B. Schmitt, Dr. R. Lanciotti
Issues to Discuss:
Surveillance
Health Department/Laboratory Infrastructure
Training
Research Priorities

ENTOMOLOGY — Long's Peak East (adjacent to main conference room)
Moderator: Dr. B. Eldridge
Rapporteurs: Dr. J. Day, Dr. R. Nasci
Issues to Discuss:
Surveillance
Mosquito Control
Health Department Infrastructure
Training
Research Priorities

November 9

0800-1200	Breakout Sessions Continue
1200-1330	LUNCH
1330-1700	Breakout Group Reports/Discussion

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Appendix B – Questions/Issues Relating to West Nile Virus in the Western Hemisphere

GENERAL EPIDEMIOLOGY/ECOLOGY

- Has West Nile (WN) virus become established (enzootic) in the Western Hemisphere?
    - What are the best surveillance methods for determining this?
    - Where geographically should surveillance efforts be focused?
- If WN virus has become enzootic, how widely has it spread in the United States.? In the Western Hemisphere?
    - What is the expected pattern of spread of the virus in this hemisphere, based on bird migration patterns and competent mosquito vector distribution?
    - How long has it been here, and how and where was it introduced?
    - Will WN virus overwinter in the Northeast?
- Which mosquito species are, or might be important potential vectors of WN virus in the United States?
- Which bird species are the principal reservoir hosts for WN virus in the United States?
- What other domestic and wild animals may be affected by WN virus?
- What roles do humans, horses, and other animals play in the transmission cycle of WN virus?
- What roles do birds or other animals play in long-term maintenance of WN virus?

PREVENTION AND CONTROL

- What is the best approach to prevent future WN virus epidemics/epizootics?
    - vector control
    - vertebrate control
    - vaccines
    - an integrated approach
- What are the most effective control methods for the potential mosquito vectors of WN virus?
- What are the infrastructure problems that need to be addressed at the state and local level to insure effective mosquito surveillance and control?
- What, if anything, should be done, during the winter months of 1999-2000, to monitor and/or control WN virus in overwintering mosquitoes?

SURVEILLANCE

- What are the best ways to enhance surveillance for WN virus in humans? In horses and other domestic animals? In wild birds? In mosquitoes?
- Can illness and death in the American crow be used as a sentinel for the presence of WN virus in a community? Other bird species?

- What kind of infrastructure problems need to be addressed to insure effective surveillance for WN virus?
- In addition to the East Coast, should enhanced surveillance for WN virus be implemented in the central and western states of the United States? In Central and South America? In the Caribbean?
- What is the best way to monitor WN virus in migrant birds?

LABORATORY DIAGNOSIS/VIROLOGY

- What laboratory methods are critical for the diagnosis of WN virus?
- How does laboratory diagnosis for arboviral diseases need to be changed to support WN virus surveillance in humans? In birds? In mosquitoes? In horses and other animals?
- What kind of infrastructure problems need to be addressed to insure adequate laboratory diagnostic support for WN virus surveillance?
- What role should local, state and regional laboratories play in supporting surveillance for WN virus?
- What biosafety considerations should be given to working with specimens suspected of containing WN virus?
- Does the strain of virus influence the pathogenesis of WN virus?

CLINICAL/PATHOGENESIS

- What is the spectrum of clinical illness of WN virus infection in humans? In horses, birds, other animals?
- What are the risk factors for severe disease in humans? In horses?
- Do humans, horses and other animals have viremia of sufficient magnitude to infect mosquitoes?

Appendix C – Guidelines for Phased Response to West Nile Virus Surveillance Data

Risk categories and phased response for West Nile virus surveillance in the United States. Local and regional characteristics may alter the risk level at which specific actions must be taken.

Risk category	Probability of outbreak	Definition	Recommended response
0	Negligible or none	Off-season; adult vectors inactive; climate unsuitable.	None required; may pursue the collection and testing of overwintering adult mosquitoes for WN virus, source reduction of overwintering adults, and public education activities.
1	Remote	Spring, summer, or fall; adult vectors active but not abundant; WN virus not detected in overwintering adult mosquitoes; WN virus not detected in host-seeking adult mosquitoes; seroconversions to WN virus not detected in sentinel hosts.	Source reduction; use larvicides at specific sources identified by entomologic survey; maintain vector and virus surveillance.
2a	Possible	Winter and spring; WN virus detected in overwintering adults.	**Implement early season emergency control plan.** Response as in category 1, plus: adulticiding of overwintering sites, e.g., sewers, underground facilities; initiate early season larval control in high-risk areas; inform public that WN virus has successfully overwintered and that personal protection measures should be taken in the early spring; persons in high-risk occupations should use personal protection at all times; initiate enhanced passive surveillance for human cases in early spring.
2b	Possible	Spring, summer, or fall; focal abundance of adult vectors; temperature adequate for extrinsic incubation; either single WN virus isolate obtained from mosquitoes or first confirmed seroconversion in sentinel hosts.	**Response as in category 1, plus:** increase larval control activities; initiate targeted adulticide use to areas of suspected virus activity to protect highly susceptible human populations; increase vector and virus surveillance; initiate enhanced passive surveillance for human cases; initiate public outreach about personal protection measures.
3	Probable	Abundant adult vectors in most areas; multiple WN virus isolates obtained from mosquitoes or avian hosts, or widespread seroconversions in sentinel hosts, or multiple equine cases, or a confirmed human case; optimal conditions for extrinsic incubation and vector survival.	**Implement emergency control contingency plan**. Response as in category 2, plus: intensify and broaden adulticiding program; expand public information program to include TV, radio, and newspapers (use of repellents, personal protection, avoidance of high vector contact areas); initiate active surveillance for human cases.
4	Outbreak in progress	Multiple confirmed cases in humans	**Continue with emergency control contingency plan:** Concentrate available resources on larviciding and on adulticiding efforts over areas at risk; hold daily public information briefings on status of epidemic; continue emphasis on personal protection measures; maintain active surveillance of vector/virus activity, human cases.

*Adapted from reference 8.

Appendix D -- Working Surveillance Case Definition of West Nile Encephalitis

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[10]. As such, it is a 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 St. Louis encephalitis (SLE) virus was the only neurotropic flavivirus with epidemic potential known to occur in the United States. 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, to a lesser extent, in neutralization tests. (To an even lesser extent, serologic cross-reactivity also occurs between these three viruses and Powassan virus, a tick-borne flavivirus endemic to the northeastern United States and eastern Canada, 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 (see Note 1) . 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 should 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 southern latitudes where the maintenance cycles of both viruses may co-exist, however, it is possible to have concurrent transmission of both viruses. In any case, epidemiologically, clinically, and in terms of prevention and control methods, the differences between these two viruses are generally subtle and largely academic.

1. 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;[2]
   - Demonstration of immunoglobulin M (IgM) antibody to WN virus in CSF by IgM-capture EIA;[3-5]
   - A $\geq$4-fold serial change in plaque-reduction neutralization test (PRNT) antibody titer to WN virus in paired, appropriately timed serum or CSF samples;[3,4,6]
   - Demonstration of both IgM (by EIA) and IgG (screened by EIA and confirmed by PRNT) antibody to WN virus in a single serum specimen.[3,5,6-7]

2. 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);
   - Demonstration of an elevated titer of specific IgG antibody to WN virus in convalescent-phase serum (screened by EIA and confirmed by PRNT).

3. 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;[4]

and/or

- A negative test for IgG antibody to WN virus (by EIA or PRNT) in serum collected ≥22 days after onset of illness.[4]

Notes:

1. Although this alone could eventually necessitate a change in the national arbovirus surveillance case definition, this will largely depend on whether WN virus persists in the United States.

2. 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 for this disease are unknown.

3. 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.

4. 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 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 1-2 months after illness onset; in a few cases it will reach undetectable levels prior to 1 month after illness onset; in a few cases it will be detectable for 2-3 months or longer, but rarely by the next arbovirus transmission season.

   - 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 both EIA 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; compared with serum, IgM antibody in CSF will be relatively short-lived.

   - 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: 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. 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. However, the P/N ratios generated by tests with WN viral antigens are generally much higher than those generated by tests with other flaviviruses.

   - Specificity of IgG EIA: WN viral IgG antibody detectable by EIA is broadly cross-reactive with all closely related flaviviruses, and this usually cannot be resolved with comparative EIAs using various flavivirus antigens.

   - 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 using a variety of other flaviviruses (owing to "original antigenic sin"), and comparative titers are often insufficiently different to implicate the homologous virus.

Based on these assumptions and 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 test results 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 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. Instead, it should usually be reserved for testing by IgM-capture EIA and possibly by other means, including virus isolation, PCR, and neutralization.

- In patients who are immunosuppressed or have been plasmapheresed, negative test results for antibody to WN virus may be false-negatives. Follow-up specimens should be collected and tested from plasmapheresed patients at least 2 weeks after plasmapheresis.

5. At CDC, serum specimens are routinely tested at a dilution of 1:400 and CSF specimens are tested undiluted. It is possible to titrate samples to determine the endpoint dilution that gives a positive P/N ratio, but this is not done routinely. Empirically, P/N ratios of $\geq$3.0 are considered positive; ratios of 2.0-2.99 are considered equivocal, and ratios <2 are considered uninterpretable if the OD of the test sample with viral antigen is <2 times the OD of the test sample 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.

6. A 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.

7. As discussed above, in a few previously infected persons, detectable IgM antibody to WN virus could theoretically persist for 2-3 months or longer, including those subclinically infected or only mildly symptomatic. Thus, in a person with a current or very recent viral syndrome such as viral encephalitis, positive test results for both IgM and neutralizing antibodies to WN virus in a single serum sample could be false-positives. In other words, although the current or recent illness may be clinically and serologically compatible with a WN viral infection, in fact its cause may not be WN virus at all. However, given the low incidence of indigenously acquired neurotropic flavivirus infections in the United States at present, this would seem to be more of a theoretical concern than a practical one. In other words, in a case of acute viral encephalitis acquired in this country, a test for IgM antibody to WN virus should have a high positive predictive value (although based on this test result alone, the patient could have SLE instead), which would presumably be even higher during an epidemic than in a sporadic case.

References:

CDC. Case definitions for infectious conditions under public health surveillance. *MMWR* 1997;46[RR-10]:12-3 (available at www.cdc.gov/epo/dphsi/casedef/enceph97.htm). See reference 10.

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.

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