WEST NILE VIRUS SURVEILLANCE PROTOCOL
2003 Season Guidance

(Note: This guidance may evolve during the season. Please make sure you have the most current recommendations.)

Public Health Action

1. Educate the public about West Nile virus, especially regarding elimination of mosquito breeding sites and use of personal protective measures.

2. Educate the public to report dead bird sightings to the local health department.

3. Educate physicians and hospital infection control practitioners to:
   a. Recognize clinical syndromes that warrant arbovirus testing (i.e. febrile headache, aseptic meningitis, or encephalitis during late summer and early fall), and
   b. Order appropriate testing for West Nile virus (WNV), La Crosse encephalitis (LAC), Eastern equine encephalitis (EEE), and St. Louis encephalitis (SLE).
      This action should be accomplished by generating a physician alert from the county health department and/or by asking infection control practitioners to assist in alerting physicians.

4. Educate veterinarians to consider West Nile virus as a possible etiology of summertime encephalitis in horses.

5. Educate government officials at all levels regarding mosquito surveillance and integrated pest management as a means of preventing cases of WNV.

6. Report total dead bird sightings (crow and non-crow) and zip code location to the West Virginia Infectious Disease Epidemiology Program (IDEP) on a weekly basis May 1 to November 30, 2003.

7. Report recently dead (< 24 hours) birds to IDEP immediately for possible testing for West Nile virus.

8. Investigate cases of human WNV and perform a visit to the homes of all confirmed WNV cases to visualize the outdoor environment, educate the family about removal of containers, mosquito habitat abatement, and use of personal protective measures, including use of mosquito repellent. Obtain latitude and longitude of the home of the case, and interview the case (or parents) to obtain information on the location of other potential exposures, including time spent out of doors during the incubation period. Include a travel history during the incubation period. Document using the Arbovirus Supplemental Investigation Form. Attach the supplemental form and all appropriate laboratory studies to the yellow card and send to the West Virginia Infectious Disease Epidemiology Program.

9. Report confirmed/probable/suspected human, equine, or avian WNV cases urgently to IDEP.
10. Given existing resources; if bird, human, or equine cases of WNV are identified:
   a. increase public education to encourage use of personal protective measures,
   b. increase public education to encourage elimination of mosquito breeding sites,
   c. increase education of government officials at all levels regarding mosquito surveillance and integrated pest management as a means of preventing additional cases,
   d. generate an alert to physicians to intensify surveillance for human cases, and
   e. generate an alert to veterinarians to intensify surveillance for equine cases.

11. If additional resources become available:
   a. establish local or regional mosquito surveillance and control capacity, and
   b. begin viral surveillance of mosquito populations.

Prevention Objectives

1. Reduce disease risk through public education to encourage:
   a. use of personal protective measures, and
   b. elimination of mosquito breeding sites.

2. If additional resources become available: reduce disease risk through development of local or regional mosquito surveillance and control capacity.

Disease Control Objectives

1. If a bird or equine case is identified: prevent the development of human cases through education of the public to use personal protective measures and eradicate mosquito breeding sites.

2. If a human case is identified: prevent the development of additional human cases through education of the public to use personal protective measures and eradicate mosquito breeding sites.

3. If positive mosquito pools are identified: prevent the development of human cases through appropriate mosquito surveillance and control.

4. If additional resources become available: prevent human cases through development of local or regional mosquito surveillance and control capacity.

Surveillance Objectives

1. Determine where West Nile virus is present in West Virginia and the extent of spread (to be accomplished through dead bird testing).

2. Estimate the intensity of West Nile virus activity, if present (to be accomplished through dead bird testing and dead bird reports).

3. Detect human and equine cases of West Nile virus, if present.
4. Determine where West Nile virus is present in West Virginia and the extent of spread (to be accomplished through adult mosquito surveillance using both CDC gravid and CDC miniature light traps).

**Public Health Significance**

For the fourth year in a row, WNV has continued to expand its territory, spreading over much of the United States. Many counties in West Virginia had birds test positive in 2002. Three West Virginia counties also had WNV-positive horses, and three positive human cases were reported from two counties. (See [www.wvdhhr.org/bph/oehp/sdc/westnile_2002_data.htm](http://www.wvdhhr.org/bph/oehp/sdc/westnile_2002_data.htm) for up-to-date Dead Bird Reports by county in West Virginia)

Public health officials must be vigilant for WNV during mosquito season because: 1) the mosquito vectors which carry WNV have been found in West Virginia, and 2) WNV-positive birds, horses and humans have been identified in West Virginia. The purpose of surveillance is to identify whether human cases are likely so that appropriate prevention measures can be taken. Public health has a major role in prevention and control of this disease through surveillance, public and provider education, and through promotion of mosquito control activities.

For reasons stated above, surveillance is an extremely important function of public health. This season, the public health community is funded to perform dead bird, mosquito and human surveillance. Equine surveillance will also be possible through collaboration with the USDA and the West Virginia Department of Agriculture. Here are the types of surveillance that should be performed and the purpose of each type of surveillance:

1. **Dead Bird Surveillance**: The purpose of dead bird surveillance is to establish whether West Nile virus is present within the jurisdiction under surveillance. This is accomplished by testing freshly dead birds for WNV. By tracking the number of dead bird reports, the intensity of viral activity can also be estimated (i.e. more dead bird reports suggests more viral activity and a higher likelihood of human cases). This information should be used to inform the public about the level of risk within the jurisdiction so they can take action to protect themselves.

2. **Mosquito Surveillance**: One purpose of mosquito surveillance is to identify mosquito breeding sites and prioritize sites for abatement. For example, sites breeding the treehole mosquito should be abated or larvacided if they are in close proximity to human populations because the treehole mosquito is the vector for La Crosse encephalitis; whereas sites breeding nuisance mosquitoes are low priority for abatement. Another purpose of mosquito surveillance is to determine if disease-carrying adult mosquitoes are present. Again, this information should be used for mosquito control so that human cases of arboviral infection can be prevented. The West Virginia Department of Health and Human Resources is conducting limited adult and larval mosquito surveillance at this time.

3. **Equine Surveillance**: The full role of equine surveillance is yet to be determined; however, horses do serve as an important indicator of WNV activity in the jurisdiction. Information on equine cases should be used to prevent human cases.
4. **Human Surveillance**: The purpose of human surveillance is to detect human WNV infection within the jurisdiction. This information should be used to prevent additional cases.

The ecology and public health aspects of WNV are complex. West Virginia public health officials are encouraged to take the necessary time to educate themselves about this disease.

**Clinical Description**

According to several recent studies most WNV infections are mild or unapparent, approximately 80% of infections are asymptomatic. About 20% of those infected develop mild febrile illness of sudden onset (West Nile fever) which is often accompanied by: malaise, anorexia, nausea, vomiting, eye pain, headache, myalgia, rash and lymphadenopathy. Symptoms are normally self-limiting and last 3 to 6 days.

Less than 1% of infections result in severe neurological disease. Neurological presentations include: Meningitis, Encephalitis, Meningoencephalitis and Acute flaccid paralysis. Signs and symptoms may include fever, weakness, GI symptoms, change in mental status, ataxia, extrapyramidal signs, cranial nerve abnormalities, myelitis, optic neuritis, polyradiculitis and seizures. A small minority of patients may develop a maculopapular or morbilliform rash involving the neck, trunk, arms or legs. Encephalitis is more commonly reported than meningitis. The most significant risk factor for developing severe neurological disease is advanced age. The case fatality rate is estimated at 10% of those with severe neurological symptoms (this equates to < 0.1% of total infections).

Preliminary data from clinical investigations conducted during the 2002 Arbovirus season are elucidating an expanding spectrum of neurological disease. Emerging and evolving clinical syndromes include: Movement disorders, Parkinsonism, Rhabdomyolysis (disintegration of muscle fibers with excretion of myoglobin in the urine) and Acute flaccid paralysis.

Movement disorders included onset of tremors and or myoclonus (usually facial involvement) > 5 days following initial symptoms. Parkinsonian-like signs were seen in both encephalitis and meningitis cases, signs included: cogwheel rigidity, bradykinesia and postural instability. Tremors were not observed at rest. Rhabdomyolysis (other causes, such as trauma and drug induced being ruled out) was identified in 14 WNV cases during 2002. Further study to assess the association are needed. The syndrome of acute flaccid paralysis usually includes asymmetric weakness without pain or sensory loss, and elevation of CSF protein and WBC. Continued surveillance and public health investigation is needed to fully define the scope of neurologic illnesses associated with WNV infection.

In the temperate zone of the world, WNV encephalitis cases occur primarily in the late summer or early fall. In the southern climates where temperatures are milder, WNV can be transmitted year round.

**Etiologic Agent**

West Nile virus is a member of the family *Flaviviridae* (genus *Flavivirus*). Other members of this family include the Hepatitis C, dengue fever, and yellow fever viruses. Serologically, it
is a member of the Japanese encephalitis virus complex that includes St. Louis encephalitis (SLE), Japanese encephalitis, Kunjin, Murray Valley encephalitis viruses, as well as others. It was first isolated in the West Nile province of Uganda in 1937. Members of the family *Flaviviridae* are single-stranded RNA viruses, approximately 40-60 nm in size.

**Reservoir**

Wild birds are the primary reservoir hosts; however, the American Crow, Blue Jay, and other corvids and raptors (hawks, owls, and eagles) are particularly susceptible. Horses, humans, and other animals are usually considered to be dead-end or incidental hosts, since they are not known to develop infectious-level viremia.

**Modes of Transmission**

The principal route of West Nile virus infection in horses, humans, and birds is through the bite of an infected mosquito. Non-infected mosquitoes can become infected through biting and obtaining a blood meal from infected birds. WNV is amplified during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and bird reservoir hosts. Infectious mosquitoes carry virus particles in their salivary glands and infect susceptible bird species during blood-meal feeding. A sufficient number of vectors must feed on an infectious host to ensure that some survive the extrinsic incubation period to feed again on a susceptible reservoir host. Even if the mosquito is infected, less than 1% of people bitten who become infected will get severely ill.

Multiple species of mosquitoes have tested positive for WNV, including: *Aedes* (4 species), *Anopheles* (6 species), *Coquillettidia* (1 species), *Culex* (8 species), *Culiseta* (2 species), *Deinocerites* (1 species), *Ochlerotatus* (9 species), *Orthopodomyia* (1 species), *Psorophora* (3 species), and *Uranotaenia* (1 species).

Of particular concern are some species of *Aedes*, *Culex*, and *Ochlerotatus* that may be more inclined to bite humans. Several species in these genera are also known cavity breeders and will breed in artificial containers.

Five additional routes of infection have become apparent during the 2002 West Nile season. It is important to note that these other methods of transmission represent a very small proportion of cases. New modes of transmission are via: Transplantation, Transfusion, Breastfeeding, Transplacental and Occupational exposures (mostly laboratory workers). (More information may be found on the CDC’s website at: http://www.cdc.gov/ncidod/dvbid/westnile/clinical_guidance.htm)

There is no documented evidence of direct person-to-person or animal-to-person transmission. There is concern that a person may get WNV from handling live or dead infected birds, so people should avoid bare-handed contact when handling dead animals, and use gloves or double plastic bags to place carcasses in garbage cans.
**Incubation Period**

The incubation period is usually three to 15 days.

**Infectious Period**

There is no direct person-to-person transmission. See section on Modes of Transmission above.

**Outbreak Recognition**

Any case of human or equine disease is defined as an outbreak during 2003. The Infectious Disease Epidemiology Program should be notified immediately upon any positive case finding in a human with WNV infection, and should also be notified of suspected infection prior to completion of testing.

WNV is confirmed in a county based on a dead bird testing positive for West Nile virus, a positive equine case, a positive human case or by demonstrating WNV infection in adult mosquitoes.

**Case Definition**

**Encephalitis or Meningitis, Arboviral**

*Clinical Description*

Arboviral infections may be asymptomatic or may result in illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes ranging from febrile headache to aseptic meningitis to encephalitis may occur, and these are usually indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis (> 5 white blood cells in CSF). Arboviral encephalitis is characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction (e.g. palsies, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements).

*Laboratory Criteria for Diagnosis*

1. Fourfold or greater change in virus-specific serum antibody titer, or

2. Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, or

3. Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA), or
4. Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g. neutralization or hemagglutination inhibition).

**Case Classification**

**Probable**: an encephalitis or meningitis case occurring during a period when arboviral transmission is likely, and with the following supportive serology:

- a. a single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or
- b. serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen.

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

**Comment**

Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g. in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with West Nile (or dengue) virus, or vice versa, in areas where both of these viruses occur.

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

- **St. Louis encephalitis/meningitis**
- **West Nile encephalitis/meningitis**
- **Powassan encephalitis/meningitis**
- **Eastern equine encephalitis/meningitis**
- **Western equine encephalitis/meningitis**
- **California serogroup viral encephalitis/meningitis** (includes infections with the following viruses: La Crosse, Jamestown Canyon, snowshoe hare, trivittatus, Keystone, and California encephalitis viruses)
- Other viral CNS infections transmitted by mosquitoes, ticks, or midges (e.g. Cache Valley encephalitis/meningitis and Venezuelan equine encephalitis/meningitis)
Laboratory Diagnosis

Human Serological Testing

It is impossible to clinically distinguish one type of encephalitis from another. Any individual in West Virginia who presents with encephalitis/meningitis during mosquito season (May 1 through November 30 in most areas of the state) should be tested for La Crosse encephalitis (LAC), eastern equine encephalitis (EEE), St. Louis encephalitis (SLE), and West Nile virus (WNV).

Serum or CSF should be sent to the West Virginia Office of Laboratory Services (OLS), 167 11th Ave, South Charleston, WV 25303 for testing or confirmation. Sherry Nestor (304-558-3530) should be contacted to arrange testing. Specimens should be accompanied by a completed Arbovirus Test Submission Form when sent to the OLS.

Detection of IgM using the antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) is optimal, and the preferred diagnostic method. Ninety-nine percent of serum and CSF specimens tested at CDC from 1999 - 2002 had detectable IgM antibody to West Nile virus in serum or CSF by the time of symptom onset. Detection of IgM antibody to West Nile virus in serum or CSF collected within eight (8) days of symptom onset is the most efficient diagnostic method. Patients who are negative for IgM antibodies in CSF or serum specimens drawn eight (8) to 21 days after illness onset are considered negative. Patients with specimens drawn within 7 days of onset of symptoms that are found negative by MAC-ELISA should have a convalescent specimen drawn at least two weeks later.

Due to the fact that IgM antibodies may persist for greater than one year, residents in endemic areas may have persistent IgM antibodies from a previous infection that is unrelated to their current illness. Since West Nile virus was present in our state last year acute (drawn within 7 days of symptom onset) and convalescent (drawn at least 2 weeks after acute specimen) serum specimen collection and submission are recommended to confirm acute infection.

When interpreting serologic tests, it must be kept in mind that close antigenic relationships of the flaviviruses may cause false positive results for WNV. Persons recently vaccinated with yellow fever or Japanese encephalitis, or recently infected with related flaviviruses (i.e. St. Louis, dengue) are likely to test positive for WNV. The plaque reduction neutralization test (PRNT) can then be used to distinguish between false-positives and serologic cross-reactivity, although some degree of ambiguity may still occur.

A four-fold increase in West Nile virus-specific neutralizing antibody titer in acute and convalescent serologies confirms acute illness.

Single serum specimens positive for WNV IgM by MAC-ELISA require confirmation by demonstration of IgG antibodies using another serologic assay (e.g. neutralization or hemagglutination inhibition). Specimens found to be positive for IgM by MAC-ELISA at OLS will be followed-up with confirmatory PRNT testing.
Dead Bird Testing

Effective July 2002, dead birds must be submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia School of Veterinary Medicine for West Nile virus testing.

The dead bird submission procedure is as follows:

1. Birds appropriate for testing include only those that have died recently (≤ 24 hours old) and have no obvious signs of trauma or decomposition (i.e. dried or missing eyes). If the carcass is soft and mushy, has an obvious odor, has skin discoloration, feathers or skin that rubs off easily, and has ants or flies, it is too decomposed for testing.

2. All species of birds can be submitted to SCWDS for testing. However, testing may be limited to crows, blue jays, and raptors (hawks, eagles, and owls) as the season progresses. If this procedure changes, all local health departments will be notified in writing by the Infectious Disease Epidemiology Program.

3. Take a cooler containing ice into the field to immediately chill the carcass(es). Use rubber gloves when picking up sick or dead animals. If gloves are not available, hands can be inserted into a plastic bag. A GIS reading should be taken, or provide latitude and longitude coordinates using a detailed county map.

4. The Infectious Disease Epidemiology Program must be contacted at 1-800-423-1271 for testing approval. If approved, a unique state identification number will be assigned to the dead bird. The bird cannot be tested without a state ID number. When a cluster of dead birds is reported, two dead birds from the cluster may be accepted for testing. When clusters of dead birds are reported, the local DNR official should be notified. DNR may wish to do additional testing.

5. Complete a “West Virginia Dead Bird Submission Form for West Nile Virus” for each bird submitted for testing. New forms can be downloaded from IDEP’s West Nile website at www.wvdhhr.org/bph/oehp/sdc/westnile.htm. Please make sure that the correct form is being used. Some information needed for each bird submitted includes the following:
   - State ID number
   - Identity and agency of person completing report
   - Date collected
   - Location (county/town/specific street address with ZIP code) including a GIS reading

   Place the paperwork and a return address label in an envelope and tape to the outside of the shipping container. If more than one bird is submitted, include information on each bird attached to the bagged bird.

6. Birds should be double-bagged in two sealed bags prior to packing.

7. Birds must be refrigerated and shipped Monday through Thursday. If the birds are collected on Friday, Saturday, or Sunday, they must be refrigerated (do not freeze)
and shipped on Monday. Carcasses should be shipped via an overnight courier (either FedEx or USPS [United States Postal Service]) in the insulated shipping containers which were provided by the Infectious Disease Epidemiology Program. A return address label must be included with the container in order for it to be returned.

8. The shipping container should be lined with a large plastic bag. Individually double-bagged birds should be packed into the container with enough blue ice packs (do not use “wet” ice) to keep the carcasses cold. Newspaper should be stuffed in spaces between the sides of the container and the outer plastic bag to keep the ice packs in contact with the birds, provide insulation, and absorb any liquid that might leak from the bags. Tape the box shut with strapping tape.

9. Send via overnight delivery to:

Dr. David Stallknecht
Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602

**DIAGNOSTIC SPECIMENS - WILDLIFE**

Note that in addition to the SCWDS address, the words DIAGNOSTIC SPECIMENS - WILDLIFE must appear on the label. This label covers federal shipping regulations. Fines may be levied if specimens are shipped and are not labeled appropriately.

10. Notify the local DNR official with the name of an individual citizen (or local health official) submitting a bird for testing as soon as possible after the bird is reported or received. Possession of songbirds is regulated by the federal government. Timely notification to DNR will keep the lines of communication open and prevent misunderstanding, as well as coordinate additional testing.

**Preventive Interventions**

There is currently no vaccine against human WNV, and no treatment.

Share these prevention messages with the public:

- Empty standing water in old tires, cemetery urns, buckets, plastic covers, toys, or any other container where mosquitoes may breed.
- Empty and change the water in bird baths, fountains, wading pools, rain barrels, and potted plant trays at least once a week if not more often.
- Drain or fill temporary pools with dirt.
- Keep swimming pools treated and circulating, and rain gutters unclogged.
- Use mosquito repellents containing DEET. Apply sparingly to children before they play out of doors, and rinse children off with soap and water when they come back in. Do not apply repellent to the face and hands of young children because they may rub it in
their eyes. Follow label directions and precautions closely.

- Use head nets, long sleeves, and long pants if you venture into areas with high mosquito populations.
- Make sure window and door screens are “bug tight.”

**Surveillance Indicators**

- Number of dead birds (crow and non-crow) submitted per county for testing for WNV.
- Proportion of weeks May to November that counties submit dead bird reports to the Infectious Disease Epidemiology Program.
- Proportion of humans with a diagnosis of encephalitis that are tested for EEE, SLE, LAC, and WNV May to November.
- Proportion of cases with complete clinical investigation: Patient demographics, involvement in outdoor activities, travel history and clinical symptoms (Part 1. of Arbovirus Investigation Form completed).
- Proportion of cases with home visit completed for environmental evaluation, including GIS coordinates of location, patient and family education (Part 2. of Arbovirus Investigation Form completed).
- Proportion of cases investigations that are totally complete: complete WV BPH Confidential Reportable Disease Case report (yellow card), complete Arbovirus Investigation form and copies of supporting laboratory results i.e. confirmatory WNV serologic or antigen results and CSF test results.