

# **GUIDELINES AND SAMPLE SUBMISSION FORM FOR INVESTIGATING SUSPECT WEST NILE VIRUS CASES IN EQUINES**

## **Introduction**

West Nile Virus (WNV) is a mosquito-borne flavivirus that was first detected in the United States in September, 1999. The virus, which can cause encephalitis, or inflammation of the brain, has been found in Africa, Western Asia, the Middle East, and the Mediterranean region of Europe. In humans, infection of otherwise healthy people usually causes a mild febrile illness or no symptoms at all. Mortality has been reported in the elderly; immunocompromised individuals also are at a higher risk.

In the U.S., the disease was isolated from neurological and other tissues from birds located at the Bronx Zoo and from crows in the New York City area. Since then, the virus has been identified in horses, humans, other mammals, mosquitoes, and wild birds in at least 45 states and the District of Columbia.

Because mosquitoes transmit the virus, it has the potential to affect livestock and poultry. Migrating birds also appear to play a role in spreading the disease. In 2002, more than 15,000 equines in 40 states were diagnosed with cases of illness caused by WNV.

Approximately one-third of the horses sickened by WNV died or were euthanized; however, the use of vaccines has reduced the rate of equine infection. While the virus may infect humans and horses, there is no documentation that infected horses can spread the virus to uninfected horses or other animals.

Limiting exposure to mosquitoes and controlling mosquito populations are fundamental in preventing the disease. The purpose of this document is to guide veterinary practitioners and field personnel in investigating and reporting suspect cases of WNV infection in equines.

## **Equine Surveillance**

WNV infection in horses may include both central nervous system and peripheral nervous system signs. These signs of disease may be indistinguishable from those produced by other equine encephalitides including rabies, equine herpesvirus-1, equine protozoal myeloencephalitis (EPM), and Eastern, Western, or Venezuelan equine encephalomyelitis. The most common signs of WNV infection in U.S. horses have been ataxia, weakness of limbs, recumbency, muscle fasciculation, and death. Fever has been detected in less than one-quarter of all confirmed cases.

### **Equine Precautions**

One of the most important considerations in prevention is to prevent exposure to mosquitoes and the use of the equine WNV vaccine. Effective mosquito control involves source reduction, i.e., the elimination of stagnant water sources where mosquitoes may breed. Insect-proofing stables and other measures that reduce exposure of equines to mosquitoes may be useful in areas of high mosquito activity.

The United States Department of Agriculture (USDA) has issued a full license to two different companies for the West Nile Virus (WNV) vaccine. The name of the vaccine is West Nile – Innovator™.

### **Human Precautions**

When working with an equine, or any other mammal showing signs of a central nervous system disorder, always take precautions to avoid exposure to rabies virus. Rabies should always be included in a differential diagnosis consideration for CNS cases. In addition, persons visiting any premises to investigate an unknown disease condition should take measures to prevent exposure to a variety of arthropod-borne zoonotic pathogens. Application of commercially available insect repellents containing DEET to clothing and to exposed parts of the body should be sufficient to protect oneself from mosquitoes carrying WNV.

### **Sample Submission**

Call Oregon Department of Agriculture (503-986-4760) or Oregon Department of Human Services (503-731-4024) for approval before submitting specimens.

Samples for laboratory analysis can be submitted to the veterinary diagnostic laboratory at Oregon State University. OSU-VDL may forward samples to the National Veterinary Services Laboratory in Ames, IA, if the need arises.

Samples for submission should be shipped via an express carrier to:

Oregon State University  
Veterinary Diagnostic Laboratory  
Magruder Hall, Room 134  
30th and Washington Way  
Corvallis, Oregon 97331  
Phone: 541-737-3261 Fax: 541-737-6817

Please report the number and type of samples and relevant epidemiological information, including location of premises (county and closest city), clinical signs observed/reported, date of onset, age of animal, outcome (alive/died/euthanized), recent travel history, and vaccination status. (**SEE FORM**, last page.)

#### **Ante-mortem Sample Collection**

Serum is the preferred sample for collection. Collect serum in a 10 mL red-top tube or clot-separator tube. Send the sample to OSU-VDL. Collection of a whole blood sample (in a 10 mL EDTA purple-top tube) is of less importance but may also be included.

Additionally, cerebrospinal fluid (CSF) may also be collected in a red-top tube labeled with the site of collection (e.g., cervical or lumbo-sacral).

#### **Post-mortem Sample Collection**

Use appropriate protective gear when collecting and processing postmortem samples (see below, "Recommendations for Safe Practices for Conducting Necropsies of Suspected WNV Cases").

If a suspect equine is to be euthanized, collect at least one serum sample in a 10 mL red-top tube or clot-separator tube, prior to euthanasia. Collection of a whole blood sample (in a 10 mL EDTA purple-top tube) is of less importance but may also be included.

### **Recommendations for Safe Practices for Conducting Field Necropsies of Suspect Animals**

Although aerosol transmission of WNV is very unlikely, precautions should be taken in laboratory and field settings. The main concern should be to prevent viral contact with open wounds and mucous membranes.

1. Keep the use of needles and sharp instruments to a minimum.
2. DO NOT use mechanical saws to obtain spinal cord samples. For proper procedures, see "Collection of Spinal Cord Segments" below.
3. Procedures that create any aerosol particles should be done in a way to minimize their dispersal.
4. Wear Tyvek® disposable coveralls or, at a minimum, a solid-front, water-resistant, long-sleeve gown.
5. Wear three pairs of gloves. The innermost pair should be latex or other disposable gloves. Substantial waterproof gloves (e.g., Playtex® kitchen gloves) should be worn over the innermost pair. The gloves should be long enough for the gown sleeves to be tucked inside the gloves; duct tape may be useful for keeping sleeves inside gloves.
6. Wear a face shield or goggles to protect mucous membranes and to avoid aerosol infection.

### **Collection of Equine Brain Tissue**

Diagrams showing the procedure for collecting equine brain tissue are reproduced from Equine Medicine and Surgery, 3rd ed., 1982, edited by Mansmann, McAllister, and Pratt (see the last page of these guidelines). Always use appropriate protective gear when collecting and processing samples.

### **Collection of CSF**

A good site to collect CSF is at the atlanto-occipital junction, just as one cuts through the ligaments prior to decapitation. Up to 15 mL of CSF can be collected from this site. Collect as much fluid as possible. CSF may also be collected from a sacral tap on postmortem.

**\* Mention of a commercial product, trademark, or brand name is for illustrative purposes only and does not constitute endorsement by any individual nor by any agency of the U.S. government.**

**Oregon West Nile Virus Surveillance  
Equine Submission Form**

**\*\*WE ONLY TEST CLINICAL CASES OF POSSIBLE WVN\*\***

**OWNER:**

Name \_\_\_\_\_  
Address \_\_\_\_\_  
City \_\_\_\_\_ State \_\_\_\_\_ Zip \_\_\_\_\_  
Phone \_\_\_\_\_ Fax \_\_\_\_\_

**SAMPLE COLLECTED BY:**

Name \_\_\_\_\_  
Address \_\_\_\_\_  
City \_\_\_\_\_ State \_\_\_\_\_ Zip \_\_\_\_\_  
Phone \_\_\_\_\_ Fax \_\_\_\_\_  
Date of Collection \_\_\_\_\_ County \_\_\_\_\_

**SIGNS/SYMPTOMS:**

ataxia / weakness	licking
anorexia (off feed)	blinking
circling	head tilt
facial twitching	hypersensitive to touch
chewing	personality/behavior changes
mental depression / sleepiness	complete paralysis
other _____	

Number of sick horses \_\_\_\_\_ Total number on premises \_\_\_\_\_  
Have horses been vaccinated? Yes \_\_\_\_\_ No \_\_\_\_\_ If yes, when \_\_\_\_\_  
Out of state travel? Yes \_\_\_\_\_ No \_\_\_\_\_  
To where? \_\_\_\_\_ Return date \_\_\_\_\_

**SPECIMENS SUBMITTED:**

\_\_\_\_\_  
\_\_\_\_\_

**RESULTS:**

Test Performed. \_\_\_\_\_  
Results \_\_\_\_\_

**Test Results Contacts:**

**Dr. Don Hansen, phone 1-503-986-4760 Dr. Emilio DeBess, phone 971-673-1111  
DHS Health Services, Acute & Communicable Disease Prevention  
800 NE Oregon St., #772, Portland OR 9723  
Phone 971-673-1111 Fax 971-673-1100**