

Suspected Case of Infectious Neurologic Disease

Primary differential diagnoses of neurologic disease; some of which can have herd health implications:

- EHV-1 myeloencephalopathy
- EEE*/WEE*/VEE encephalomyelitides
- WNV* encephalitis
- Rabies
- Botulism
- Tetanus
- Equine protozoal myeloencephalitis

A wide range of infectious and non-infectious diseases can give rise to neurologic signs in the horse.

The horse is a dead-end host of EEE, WEE and WNV viruses. If any of these diseases occurs, it indicates infected vectors are present in an area and there is increased risk of viral exposure for a susceptible population.

Establish Biosecurity Perimeter

(Note: Until proven otherwise, respond to 'worst-case' scenario(s): Equine Herpesvirus myeloencephalopathy (EHM), Rabies, VEE and EEE.)

Identify Primary Biosecurity Perimeter

The primary perimeter is centered on the location of the disease case(s); it should be extended until a barrier to prevent further spread of infection is identified.

The primary perimeter may encompass the entire equine facility (farm, showground or racetrack), or if site design permits, the perimeter may only contain part of the equine facility (barn/paddock). The perimeter should be clearly defined by physical barriers. Signs should be used to identify the perimeter and control access.

Note: More than one primary perimeter may be established if case development warrants and facility design permits.

The primary perimeter contains all suspect infected animals and animals in immediate contact with them.

All animals within the primary perimeter should be considered infected or exposed to infection and potentially contagious until the outbreak is declared over. Animals are prohibited from exiting the primary perimeter, and biosecurity measures are implemented to prevent the risk of infectious agents leaving the area.

If the equine facility has an appropriately designed and managed isolation facility then the primary perimeter will be around this facility.

If the affected horse was moved from its barn to the isolation facility, a primary biosecurity perimeter must be maintained around the barn from which the affected horse originated.

While most causes of neurological disease in the horse are not contagious, those that are can result in widespread exposure before agent identification. A primary perimeter should be immediately established under the following conditions:

- Multiple febrile animals (+/- respiratory disease) or a horse with neurological disease.
- Concomitant fever and neurologic signs in multiple horses.
- In addition, immediate removal to an isolation facility of any horse with fever and neurological signs is recommended.

Implement Primary Perimeter

- *Stop horse movement.*
 - Affected horses should be moved to a separate isolation facility or confined to their stalls.
 - Clinically unaffected horses are confined within the primary perimeter and managed to minimize spread of an infectious agent.
- *Disease surveillance*
 - Record rectal temperatures twice daily
 - Physical inspections for clinical signs
- *Limit human movement*
 - Access is limited to essential personnel only—veterinarians/technicians/caretakers.
 - All personnel follow biosecurity protocols.Security personnel may be employed at perimeter access points
- [Biosecurity Guidelines](#)

Identify Secondary Perimeter

If the primary perimeter does not encompass the entire facility, it is appropriate to establish a secondary perimeter that does. All animals within the secondary perimeter are considered free of infection, but at increased risk of exposure, making enhanced disease surveillance and contagion control measures necessary.

Animals should be allowed to move into and out of the secondary perimeter only from outside the equine facility, and under the control of the veterinarian in charge.

- *Increase disease surveillance*
 - Monitor and record rectal temperatures of all horses twice daily
 - Physical inspection for clinical signs

Note: It may be advisable to have these tasks performed by individuals designated by the official veterinarian or event management as opposed to representatives of individual horsemen.
- *Regulate horse movement*
 - Record Arrival/departure information including:
 - Date
 - Origination/Destination
 - Carrier information
 - Establish health requirements for:
 - Access to secondary perimeter from outside facilities
 - Health certificate w/disease specific endorsement
 - Vaccination recommendation/requirement
 - In the absence of a specific diagnosis, *recommendations* may be more appropriate than *requirements*.
 - Exit from secondary perimeter to outside facilities:
 - Health certificate w/disease specific endorsement
 - Vaccination requirements (disease dependent)
 - Testing requirement (disease dependent)

Note: Exit health requirements should be established consensually with representatives of recipient facilities/jurisdictions/states. (A meeting or conference call can be an effective method of establishing consistent policy amongst recipients).

Communication

I. Event Management

- Physical plant modification instructions
 - Barriers—designation and establishment of physical perimeter
- [Biosecurity Guidelines](#)
 - Disinfection instructions
 - During outbreak
 - Before restocking facility with healthy horses
 - Waste removal
 - Vermin control -- Insect control when arbovirus (WNV/EEE/WEE/VEE) infection is suspected or confirmed
- Personnel Management
 - Requirements
 - Instructions
 - Notification of zoonotic risk, if pertinent
- Outbreak updates
- Event Management Biosecurity Resources:
 - [Biosecurity Tool Kit for Equine Events](#)

II. Veterinarians

- Instructions—disease surveillance/testing/reporting
- Health requirements—entrance into/exit out of facility
- Outbreak updates

III. Horsemen

- Disease information for horsemen/owners
- [Biosecurity Guidelines](#)
- Human exposure/zoonotic risk management
- Instructions for caretakers
 - [Instructions for caretakers](#)
 - Notification of zoonotic risk, if present
 - Instructions for reporting human disease
- Outbreak updates

- Requirements for equine entrance into/exit out of facility

IV. Regulatory Agencies

- Disease notification
 - Veterinarians are advised to be aware of currently reportable diseases either to the USDA (federal area veterinarian in charge) or to the State
 - Veterinarian and (if applicable) also abide by federal regulations

Note: State and USDA veterinarians can be useful resources during outbreaks of non-reportable infectious disease.
 - Outbreak updates

V. Media

- Dissemination of information to horsemen and appropriate industry groups:
 - Outbreak updates
 - Requirements for equine movements into/export out of facility

VI. Related Industries

- Outbreak updates
- Summary of biosecurity measures
- Requirements for equine movements into/ out of facility

Attempt Diagnosis

Complete physical/neurologic exam

Diagnostic Sampling, Testing and Handling

CBC/Chemistry Panel + Blood ammonia (r/o hepatoencephalopathy)

Virus Isolation – inoculation of appropriate samples into cell culture and identification of any agent causing cytopathic changes. Virus isolation is not always the most sensitive and will take at least 2-5 days to get a result depending on the laboratory, amount of virus in a sample and cell systems used.

Immunoassay – detects viral antibodies by ELISA.

Sensitive and quick but not always specific: results should be available in 1-2 days.

In most cases the sample of choice is serum, but for WNV, EEE, WEE, and VEE viruses, the assay can also be performed on CSF.

PCR—detects viral or bacterial nucleic acid (DNA or RNA), is highly sensitive in being able to detect small amounts of DNA/RNA, and offers rapid lab turnaround time (1-2 days).

Note: PCR tests cannot differentiate between live organisms and nucleic acid from killed bacteria/ inactivated viruses. Therefore PCR testing for viral/ bacterial pathogens should be done in conjunction with culture/ attempted virus isolation in cell culture.

Antibody quantitation – determined using various serologic tests (e.g viral neutralization, complement-fixation, hemagglutination inhibition etc.). These tests usually require paired (acute and convalescent) sera collected at a 2-3 week interval; in most instances they provide a retrospective diagnosis of the cause of a disease outbreak.

Cerebrospinal Fluid Analysis—(cytology, total protein, color) may be useful in narrowing a differential diagnoses. Cerebrospinal fluid analysis may indicate a viral meningitis/ encephalitis/ myelitis on the basis of increased WBC (> 7 cells/ul) and total protein (>70 ug/dl).

	EEE	WEE	WNV	Rabies	EHV	Botulism	EPM	Parasite
Protein	↑	↑	N to ↑	N to ↑	↑↑↑	N	Mod incr. > 80 mg/dl	↑↑
Cells	↑↑	N or ↑	N To ↑↑	N to ↑	N	N	N To ↑	↑↑
Cell	P, L	L	L	L	L	N	L	P, E
Color			(Xantho)	(Xantho)	Xantho		(Xantho)	

N=Normal P=PMN L=Lymphocyte M=Monocyte E=Eosinophil

Laboratory Selection

Identify laboratories and their respective testing capabilities prior to need, so the clinician can get the desired results in an acceptable time frame. Be sure the lab you select will be able to do the desired testing and get the results to you in a timely manner. There are a number of quality institutional and private labs available.

Sampling

Before sample collection, contact the laboratory for information on what samples should be collected, how they should be handled and shipped to the laboratory.

The following can be applied to most situations where a virus infection is suspected:

All swabs and small tissue specimens for viral isolation should be placed in individual tubes of viral transport medium (VTM).

Swabs should be placed in 2-4 ml of VTM. Larger volumes of medium should not be used because of the dilution effect. While VTM is commercially available, **the simplest approach is to purchase kits each containing 1-2 swabs, and a vial containing VTM. A list of suppliers is provided below.**

Swab tips should be sterile Dacron (polyester) with plastic or aluminum shafts.

Avoid swabs with wooden shafts and cotton wool or calcium alginate tips for viral isolation; these materials interfere with isolation and may also prove inhibitory in the case of PCR testing.

Samples of respiratory tract secretions can be obtained using swabs that can range from 6 to 18 inches in length; Effective sampling of the equine nasopharynx will require 16-18 inch swabs, however, passing the latter type of swab can be resented by some horses, especially younger animals. (Note: No comparison study has been carried out on the respective reliability of the different types of swabs for the detection of different equine respiratory pathogens)

Use of inappropriate sample collection materials can compromise reliability of test results!

For CSF sampling:

Either short (6 cm) or long (15-30 cm) spinal needles.

Sedation or short-term anesthesia (depending on collection site) is likely required

Sample collected by syringe and transferred into EDTA and serum tubes (without wax).

Suppliers

FischerScientific (www.fischersci.com); type “viral transport” into the search box for several choices.

Hardy Diagnostics, Santa Maria, CA (805 346-2766 ext.5658) or (www.hardydiagnostics.com).

Sample Collection

Nasal/ nasopharyngeal swab or nasal washings collected into viral transport medium for both viral isolation and viral nucleic acid detection by PCR assay and/or of viral antigen detection by antigen capture ELISA.

In collecting a specimen of respiratory secretions, the swab should be passed along the ventral meatus and allowed to remain in place for at least 10 seconds before withdrawal.

Immediately place specimens in VTM in a cooled container; they should be kept cold prior to transport to the laboratory. (In some disease situations it may be advisable to submit more than one swab per horse.)

Blood sample (EDTA or acid-citrate dextrose (ACD) tubes) for:

PCR assay for detection of EHV-1 nucleic acid

IgM Capture ELISA test for EEE, WEE, WNV antibody determination

Cerebrospinal Fluid— *Click here for CSF fluid collection document.*

Most viruses are heat-labile and are inactivated within minutes at 140 °F (60 °C) and within hours at 98.6 °F (37 °C).

Specimens should be refrigerated immediately after collection and hand carried or express-shipped to ensure reaching the laboratory in a refrigerated condition.

If a delay of more than 48 hours is expected between specimen collection and laboratory submission, specimens other than unclotted blood should be packed in individual plastic bags and frozen immediately. Where EHV-1 infection is suspected, specimens should be kept at 4 °C or on dry ice, but not at -20 °C. Acute phase sera should be saved and frozen at -20 °C.

If dry ice is used for freezing specimens, the samples must be kept in airtight plastic bags or sealed containers; CO₂ released from dry ice is harmful to most viruses

Serum sample

Such samples can be tested later. Acute and convalescent samples should be tested together if other diagnostic tests techniques have failed. Although this information may not be immediately useful for managing a disease outbreak, it may more helpful in the assessment of future risk or in the evaluation of a vaccination program.

Cerebrospinal fluid for cytology, virus isolation and PCR

For cell counts and protein, place sample in EDTA tube.

For PCR or viral culture, place sample in EDTA tube.

For measurement of disease specific antibody, place sample in a serum tube without the wax separator.

Post-mortem tissue samples: (formalin fixation and fresh, chilled samples)

A rabies protocol should be followed on ALL horses exhibiting signs of neurologic disease which undergo a post-mortem examination. Certain infectious causes of viral neurologic disease in the horse are likely transmissible to humans.

Note: Post-mortem sample collection requires observance of appropriate precautions at time of collection to avoid possible human exposure. *Link to necropsy procedure for suspected cases of zoonotic disease document*

Brain tissue—*Link to removal of the brain document*

Spinal cord

CSF fluid

Sample Transportation

Contact the laboratory for information on the preferred shipping protocol for certain types of specimens, hours of operation for receiving shipments, and whether a laboratory is open over weekends or on holidays for receipt of diagnostic materials.

Use the appropriate submission form provided by the laboratory (FAX or internet download).

All samples for viral isolation must be shipped cold (in an insulated container with cold packs), and preferably arrive within 24 hours of dispatch. Always use overnight or same-day delivery services.

Frozen samples must be shipped on dry ice or several frozen freezer packs and appropriate packaging. (Check with commercial shipping company for specific shipping requirements; noncompliance can result in the package being rejected.)

If at all possible, do not ship on Fridays; not every lab is open to receive samples on weekends. Refrigerate or freeze samples and ship on the following Monday.

Virus containing samples are considered hazardous and must comply with IATA guidelines for air shipping or Postal Service guidelines (see below).

For local or in-state laboratories, a courier service may be more expedient and less complicated than using a commercial shipping company. (Notify lab if courier service is being used and determine specifically where and to whom sample is to be delivered.)

Safe shipping of samples:

For shipping by air, call FedEx or alternative company Dangerous Goods/Hazardous Materials Hotline. The number to call in the case of FedEx is 1-800-463-3339 (press 81) for further information.

The **United States Postal Service** has set specific guidelines for the proper packaging of biological materials for shipment. Diagnostic specimens, potentially infectious specimens, and other animal products

are considered hazardous materials. Shipping services may refuse to handle any package that shows signs of internal breakage, spillage, or dampness. The sender could be held legally responsible for improperly packaged specimens; careful packaging is essential.

Shipping guidelines:

Submit all specimens in a leak proof container.

Enclose completed submission forms in a separate plastic bag and place between the inner sample container and the outer shipping container.

Surround that container with sufficient absorbent material to absorb any possible leakage.

Containers must then be enclosed in a sturdy and sealed secondary container (cardboard, plastic, styrofoam, etc.).

If more than one primary container is placed in the secondary packaging, each container must be wrapped with enough absorbent material to ensure that contact is prevented and that the absorbent material can absorb the entire contents of all materials being shipped.

Fresh tissue samples should be placed in individual, well sealed, heavy plastic bags or other containers. Double bag to prevent leakage.

Ship refrigerated and frozen specimens with adequate cold packs to ensure samples are kept cool or frozen during shipment.

Do not:

- submit samples in syringes
- include needles in samples submitted
- use ice cubes or water filled plastic bags as refrigerant
- wrap submission form(s) around sample(s)

Diagnosis

Proceed based on disease-specific information:

- EHV-1 (link to EHV-1 doc)
- WNV (link to WNV doc)
- EEE/WEE/VEE (link to this doc)
- EPM (link to EPM doc)

No Diagnosis

- Maintain biosecurity measures for 21-28 days after onset of last clinical case

Click here for
Expanded Differential Diagnoses

- Consult infectious disease expert