

Suspected Case of Contagious Infectious Neurologic Disease

Primary differential diagnoses for neurologic disease having herd health implications:

EHV-1
 EEE*/WEE*/VEE
 WNV*
 Rabies
 Botulism

Although the horse is a dead-end host, disease occurrence indicates infected vectors are present and increased risk of disease exposure exists for susceptible population.

Establish Biosecurity Perimeter

Until proven otherwise, respond to 'worst-case' scenario(s): Equine Herpes Virus / Rabies.

Identify Primary Biosecurity Perimeter

The primary perimeter is centered on the location of the neurologic disease case(s), and should be extended until a barrier to 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 suspected infected animals and animals in immediate contact with them.

All animals within the primary perimeter should be considered infected and contagious until the outbreak is declared over. Animals are prohibited from exiting the primary perimeter, and biosecurity measures are implemented to prevent 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) and a horse with neurological disease

Concomitant fever and neurologic disease 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 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 Protocols](#)

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 travel into and out of the secondary perimeter only from outside the equine facility, and under the regulation 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 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-- Maximize insect control when arbovirus (WNV/EEE/WEE/VEE) infection is suspected or confirmed
- Personnel Management
 - Requirements
 - Instructions
 - Notification of zoonotic risk, if present
- Outbreak updates

II. Veterinarians

- Instructions— disease surveillance/testing/reporting
- [Biosecurity Guidelines](#)
- 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 Grooms link to document](#)
- 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 to the State veterinarian and abide by State regulations
 - State and USDA veterinarians remain useful resources during outbreaks of non-reportable infectious disease.
- Outbreak updates

V. Media

- Dissemination of information to horsemen and related industry members:
 - Outbreak updates
 - Requirements for equine import into/export out of facility

VI. Related Industries

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

Attempt Diagnosis

Complete physical/neurologic exam

Tests

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

Virus Isolation – inoculation of sample in tissue culture and identification of any resultant viral growth. This type of test is not always the most sensitive and will take at least 2-5 days to get a result depending on the laboratory and the amount of virus in the sample.

Immunoassay – detects virus specific antibodies through ELISA tests.

Sensitive and quick, results may be available in 48 hours.

In most cases the sample of choice is serum, but tests for several pathogens, WNV, EEE, WEE, and VEE can be performed on CSF.

PCR—detects viral or bacterial nucleic acid (DNA or RNA), is highly sensitive to small amounts of DNA/RNA, and provides rapid lab turn around time (<48 hours).

Note: PCR tests cannot differentiate between live bacteria and DNA from denatured or dead.

Therefore PCR testing for bacterial organisms should always be done in conjunction with culture.

Antibody titers – using various serology tests (e.g viral neutralization, hemagglutination inhibition etc.). These tests usually require two samples collected at a 2-3 week interval, and are unlikely to provide a diagnosis during an ongoing disease outbreak.

Cerebrospinal Fluid Analysis—(cytology, total protein, color) may be useful in narrowing differential diagnoses. Cerebrospinal fluid analysis may indicate a viral encephalitis 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. Some laboratories are able to offer a wide array of diagnostic tests by forwarding received samples to other laboratories. In time sensitive situations, diagnostic test results can be expedited by submitting samples directly to the laboratory that will actually be performing the test.

The laboratory should be accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). Laboratory information is available at www.aavld.org/. Click on the Laboratory Directory: www.aavld.org/aavld-3/lab.jsp

Testing Supplies

Contact the laboratory for sample requirements before sample collection.

The following can be applied to most viral sampling situations:

All swabs and small tissue specimens for viral isolation should be placed in viral transport media (VTM).

Swabs should be placed in 2-3 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.**

Swabs should be made of sterile Dacron (polyester) with plastic or aluminum shafts.

Avoid swabs with wooden shafts and/or calcium alginate swabs for viral isolation; these materials interfere with isolation and can also inhibit PCR testing.

Cotton swabs can be used, but tend to absorb viruses such as influenza thereby reducing test sensitivity.

Swabs need only be 6 inches long in order to reach far enough into the nose for a good sample. The long proctoscopic swabs, or home-made swabs that can reach the nasopharynx are unnecessary and may be strongly resented by horses!

Use of inappropriate sample collection materials will 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 placed 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 swab collected into viral transport medium for both viral isolation and/or detection by immunoassay or PCR.

Collect the swab from the ventral meatus, ensuring enough restraint for the swab to be held against the mucosa for 2-3 seconds.

Immediately place in a cooled container prior to transport to the laboratory. (Some labs prefer more than one swab per horse, so no harm in sending two separate swabs.)

Blood sample (EDTA tubes) for:

PCR for detection of EHV-1 antigen

IgM Capture ELISA test for EEE, WEE, WNV antibodies

Cerebrospinal Fluid— [Click here for CSF fluid collection document.](#)

Most viruses are extremely 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 should be packed in individual plastic bags and frozen immediately.

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 viruses

Serum sample, save and freeze.

This sample can be used later in combination with a convalescent sample for serological diagnosis if other techniques have failed. Although this information may not be immediately useful for managing the disease outbreak, it may aid 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 for ALL horses demonstrating encephalitis that undergo a post-mortem.

Most causes of viral encephalitis in the horse are also zoonotic for humans.

Note: Post-mortem sample collection requires appropriate precautions to avoid 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 Transport

Contact the laboratory for information on their preferred shipping methods, and hours of operation for receiving shipments.

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

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

Frozen samples will require dry ice and appropriate packaging. (Check for shipping requirements; noncompliance will result in the package being rejected.)

Do not ship on Friday; few labs receive samples on weekends. Refrigerate samples and ship on Monday.

Viral 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 commercial shipment. (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 air shipping call FedEx Dangerous Goods/Hazardous Materials Hotline at 1-800-463-3339 (press 81) for further information.

The **United States Postal Service** has set specific guidelines for the proper preparation 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.

Some 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
- send needles in samples
- use ice cubes or water filled plastic bags as coolant
- wrap submission form(s) around sample(s)

Diagnosis

Proceed based on disease-specific information:

- [EHV-1](#)
- [WNV](#)
- [EEE/WEE/VEE](#)
- [EVA](#)

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