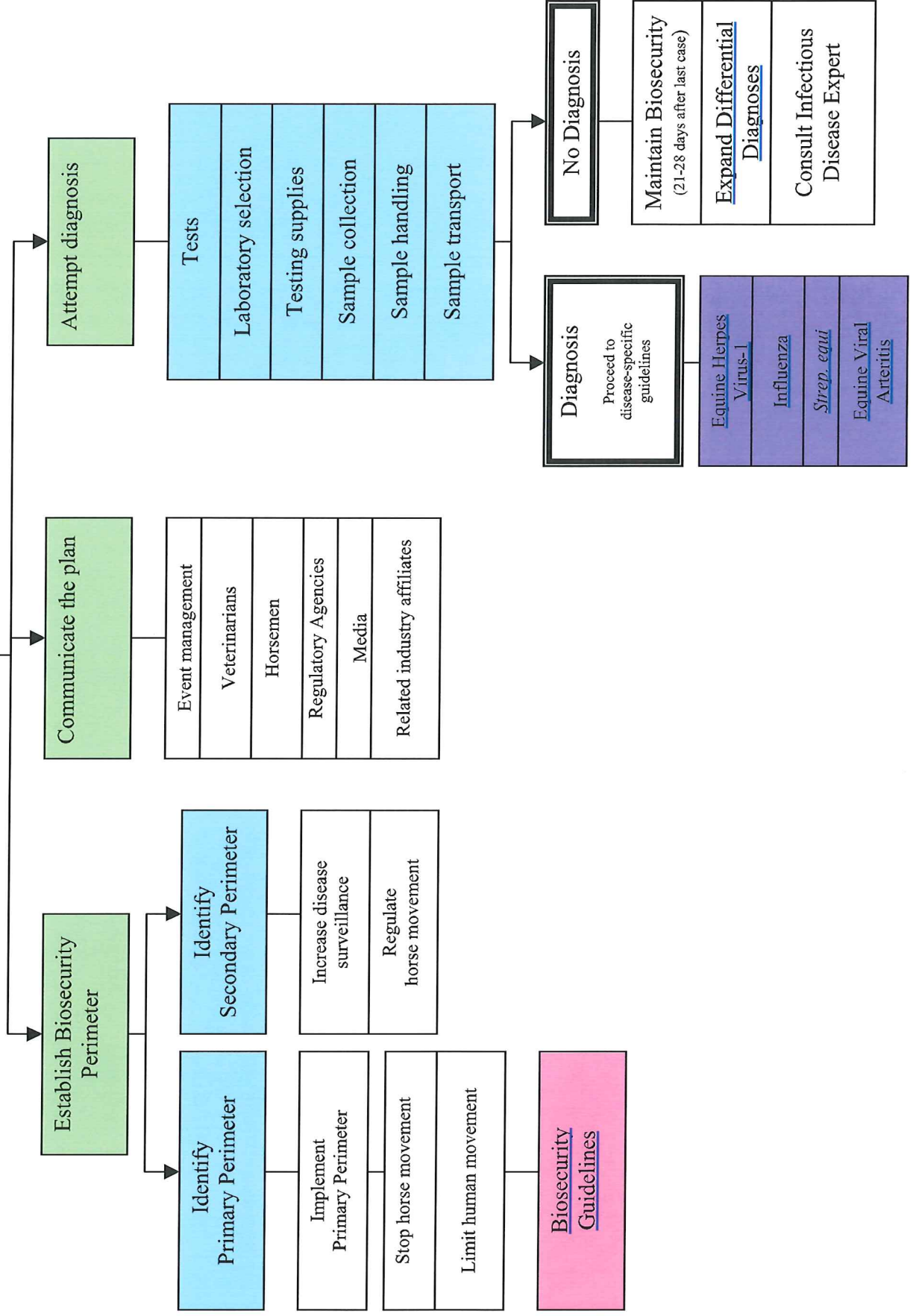


# Suspected Case of Infectious Respiratory Disease



## Suspected Case of Contagious Infectious Respiratory Disease

### *Clinical Signs:*

- Fever
- Cough
- Lymphadenopathy
- Abnormal nasal discharge
- Abnormal respiratory sounds
- Urticaria, limb edema

*Fever presenting alone should be considered as suspected contagious respiratory disease until proven otherwise.*

*Absence of fever, in the presence of respiratory clinical signs, does not rule out contagious infectious disease.*

Primary differential diagnoses for respiratory disease having herd health implications:

- Influenza
- Equine Herpesvirus-1 (EHV-1)
- Equine Herpesvirus-4 (EHV-4)
- Equine Viral Arteritis (EVA)
- Streptococcus equi*

## Establish Biosecurity Perimeter

The primary perimeter is centered on the location of the contagious respiratory disease case(s), and 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.

*Equine influenza virus spreads effectively as an aerosol over distances up to 50 yards, and the common air space of barns will mean that all horses in that barn may be infected.*

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.

Until proven otherwise, respond to 'worst-case' scenario(s):

Influenza virus (direct transmission--aerosol)

*Strep. equi* (indirect transmission—fomites)

## Implement Primary Biosecurity Perimeter

2

- *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 are 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, which 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, vaccination recommendations may be more appropriate.*)
    - Exit from secondary perimeter to outside facilities:
      - Health certificate w/disease specific endorsement
      - Vaccination requirements (disease dependent)
      - Testing requirement (disease dependent)

While testing of unaffected, unexposed horses may not be necessary in all circumstances, 'consumer confidence' may be increased by the existence of diagnostic test results supportive of declaration of absence of clinical disease.

**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
- 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](#)
- 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

- Provide information that assists in risk management and determination of risk aversion:
  - Outbreak updates
  - Summary of biosecurity measures implemented
  - Requirements for equine import into/export out of facility

### Attempt Diagnosis

#### Tests

**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 require a *minimum* of 2-5 days for results.

**Immunoassay**--detects viral proteins through ELISAs or fluorescent antibody tests.

These tests are sensitive and quick, and results may be available in 48 hours.

Currently, the only test that can be run either stall-side or in-office, is a rapid immunoassay to detect **influenza virus**. (An example is the Becton Dickinson Directigen™ Flu A Test Kit which provides results in 15 minutes.)

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

**Note:** PCR tests cannot differentiate between live bacteria and DNA from denatured or dead organisms. 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.) and usually requiring two samples collected at a 2-3 week interval. Cross reactivity with vaccine derived antibodies may obscure interpretation of results. Antibody titers may be of questionable use in establishing a diagnosis during an ongoing disease outbreak.

**Bacterial culture and sensitivity**—Use culturette labeled for bacterial sampling, only. Viral collection swabs are **not** interchangeable with bacterial collection swabs.

As clinical differentiation of pathogens is difficult, the best testing strategy is to take samples that will allow for both culture of the pathogen and detection by immunoassay or PCR. The laboratory may initially test a sample using rapid, sensitive immunoassay or PCR, and if positive the sample can then be used for viral isolation.

**Test for all likely pathogens**—typically Influenza, EHV-1 &4, and EVA +/- *Strep equi*.

Virus isolation: Influenza, EHV 1&4, EVA

PCR: Influenza, EHV 1&4, EVA, *Strep equi*

Bacterial culture: *Strep equi*

Some laboratories offer ‘respiratory panels’ which can reduce testing costs. The Lucy Whittier Molecular and Diagnostic Core Facility at UC-Davis (<http://www.vetmed.ucdavis.edu/vme/taqmanservice/>) offers a diagnostic panel that includes EHV-1&4, influenza, *R. equi*, *S. equi*, EVA; cost is \$75.00

### 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/](http://www.aavld.org/). Click on the Laboratory Directory: ([www.aavld.com/aavld-3/lab.jsp](http://www.aavld.com/aavld-3/lab.jsp))

### Testing Supplies

Contact the laboratory for sample requirements before sample collection.

For 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 bacterial culture, nasopharyngeal washes provide the most comprehensive sample:

Sterile, single-use 8-10 fr. Polypropylene catheters

Sterile saline

60 ml syringes

Sterile, lidded plastic container (urine collection jar)

#### Suppliers

FischerScientific ([www.fischersci.com](http://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](http://www.hardydiagnostics.com)).

## Sample Collection

Timing of sample collection can affect test results.

Viral shedding begins the first day of clinical signs; sample early in the course of disease.

*S.equi* shedding typically begins 24-48 hours after onset of fever; do not sample too early.

Sample collection in all cases of suspected infectious respiratory disease:

Wear disposable exam gloves; change after each horse

Disinfect twitch, lead shank, lip chain after each horse

In live horses the following samples should be collected:

**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.)

**EDTA blood sample** for detection of EHV-1 (some labs prefer heparin). Cool, but do not freeze.

#### **Nasopharyngeal wash**

Pass a sterile polypropylene catheter (8-10 fr.) through the ventral nasal meatus until resistance is met. Flush 60 ml of sterile saline through the catheter. Catch reflux fluid that drains from the nostrils into sterile container. Refrigerate sample; do not freeze.

If washes are being performed on multiple horses, exercise caution to avoid cross-contaminating the exteriors of collection containers.

**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.

Most viruses are extremely heat-labile and are inactivated within minutes at 60 °C (148° F) and within hours at 37 °C (98.6° F). 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; CO<sub>2</sub> released from dry ice is harmful to viruses

**Post-mortem tissue samples:**

Collect small blocks of lung tissue (1-2 cm<sup>3</sup>) into viral transport medium vials with the swabs removed, or placed in sealed bags or vials without viral transport medium. Immediately place in a cooled container prior to transport to the laboratory.

## Sample Transport

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

Use the correct submission form for the laboratory as provided by FAX or downloaded from the web.

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

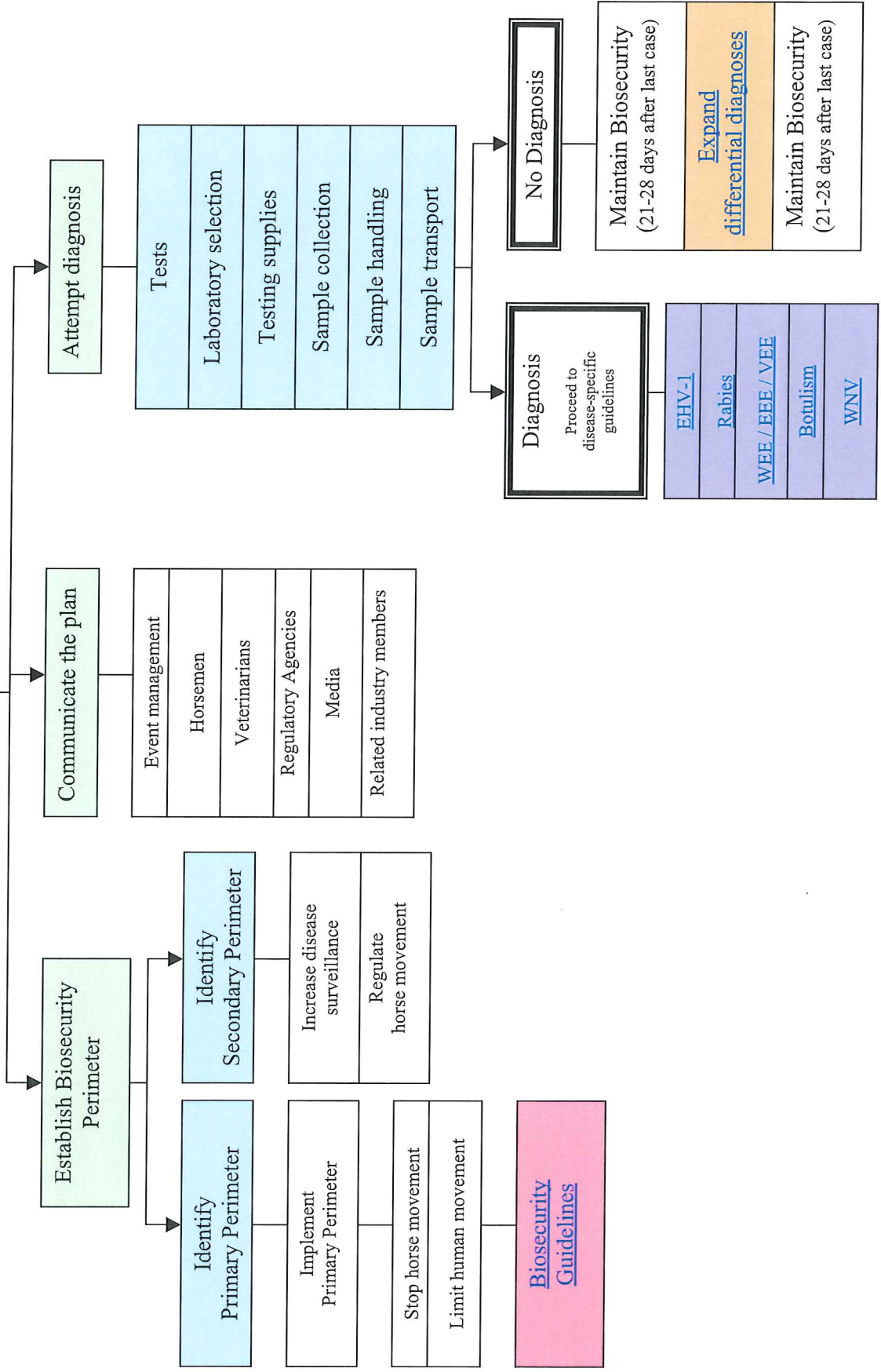
Proceed based on disease-specific information:

- [Influenza](#)
- [EHV-1](#)
- [Strep. equi](#)
- [EVA](#)

## No Diagnosis

- Maintain biosecurity measures for 21-28 days after resolution of last clinical case.
- Daily treatment logs should be maintained for all horses housed within the primary perimeter—whether or not having been clinically affected by the contagious respiratory disease.
- Expand diagnostic testing.
- Consult infectious disease expert.

# Suspected Case of Infectious Neurologic Disease



Attempt diagnosis

- Tests
- Laboratory selection
- Testing supplies
- Sample collection
- Sample handling
- Sample transport

No Diagnosis

Maintain Biosecurity (21-28 days after last case)

Expand differential diagnoses

Maintain Biosecurity (21-28 days after last case)

Diagnosis

Proceed to disease-specific guidelines

- EHV-1
- Rabies
- WEE / BBE / VEE
- Botulism
- WNV

Communicate the plan

- Event management
- Horsemen
- Veterinarians
- Regulatory Agencies
- Media
- Related industry members

Establish Biosecurity Perimeter

Identify Secondary Perimeter

- Increase disease surveillance
- Regulate horse movement

Identify Primary Perimeter

- Implement Primary Perimeter
- Stop horse movement
- Limit human movement

Biosecurity Guidelines

## 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](http://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](http://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; CO<sub>2</sub> 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)

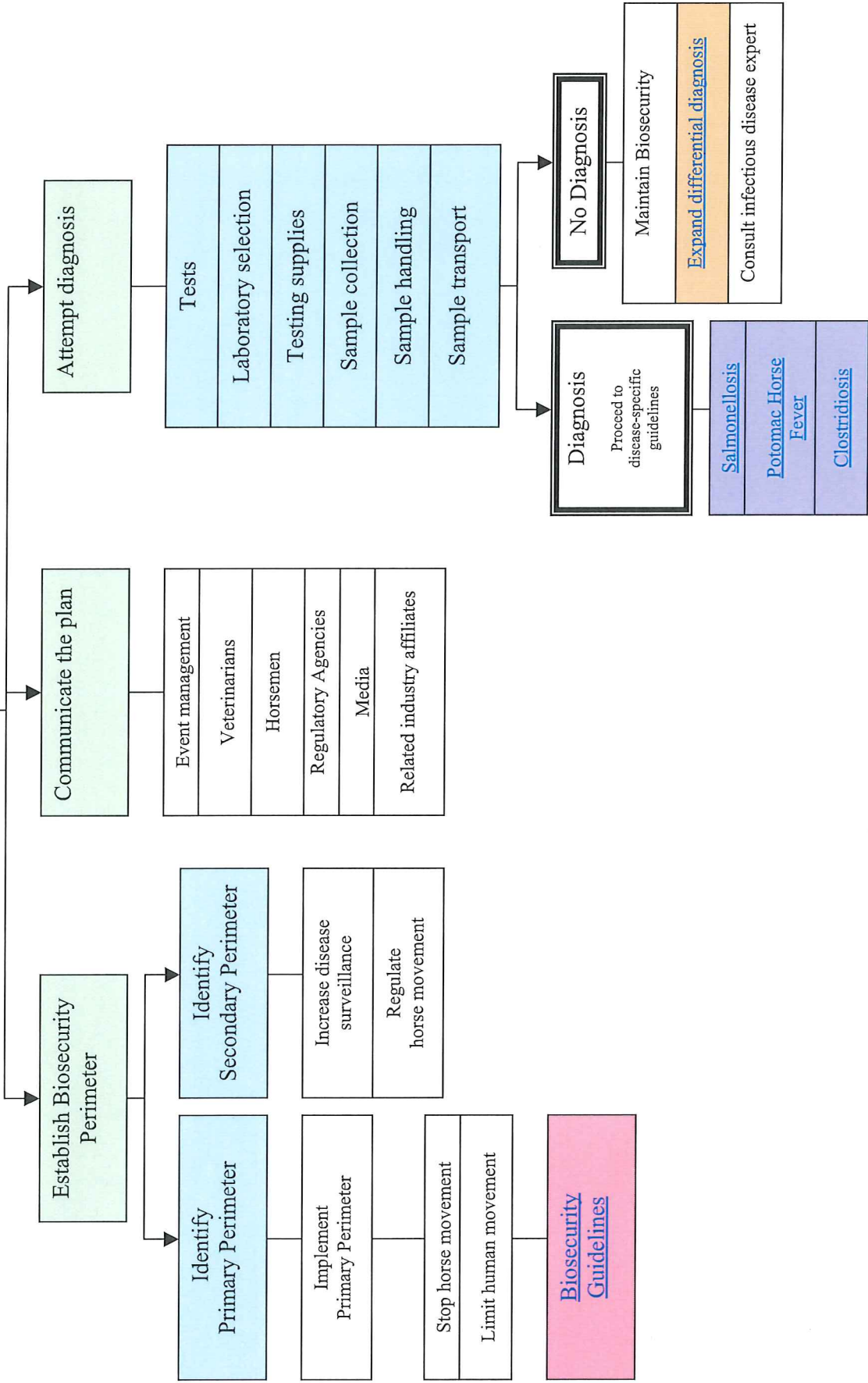
**No Diagnosis**

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

Click here for  
Expanded Differential Diagnoses

- Consult infectious disease expert

# Suspected Case of Infectious Diarrheal Disease



Attempt diagnosis

- Tests
- Laboratory selection
- Testing supplies
- Sample collection
- Sample handling
- Sample transport

No Diagnosis

- Maintain Biosecurity
- Expand differential diagnosis
- Consult infectious disease expert

Diagnosis  
Proceed to disease-specific guidelines

- Salmonellosis
- Potomac Horse Fever
- Clostridiosis

Communicate the plan

- Event management
- Veterinarians
- Horsemen
- Regulatory Agencies
- Media
- Related industry affiliates

Establish Biosecurity Perimeter

Identify Secondary Perimeter

- Increase disease surveillance
- Regulate horse movement

Identify Primary Perimeter

- Implement Primary Perimeter
- Stop horse movement
- Limit human movement

Biosecurity Guidelines

## Suspected Case of Infectious Diarrheal Disease

### Signs:

soft to watery feces  
+/- fever

### Primary infectious differential diagnoses with herd health implications:

Salmonella spp.  
Clostridial enteritis  
Potomac Horse Fever

## Establish Biosecurity Perimeter

The primary biosecurity perimeter is centered on the location of the contagious disease case(s) and exposed animals. It is 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 an isolation facility, a primary biosecurity perimeter should remain around the barn from which the affected horse originated.

Until proven otherwise, respond based on 'worst case' scenario: *Salmonella*.

## 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:*
  - Monitor horses' fecal consistency
  - Record rectal temperatures twice daily

Note: It is preferable not share thermometers among horses; each horse should have its own thermometer. Alternatively, thermometers should be thoroughly cleaned and disinfected (w/ isopropyl alcohol) between uses.

- *Limit human movement.*
  - Access is limited to essential personnel only—veterinarians/technicians/caretakers.
  - All personnel follow biosecurity protocols.
- [Biosecurity Protocols – Click here for document.](#)

## Identify Secondary Perimeter

If the primary perimeter does not encompass the entire facility, it is appropriate to establish a secondary perimeter which does. All animals within the secondary perimeter are considered free of infection, but are 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.

- *Increased disease surveillance*
  - Monitor horses' fecal consistency
  - Record rectal temperatures twice daily
- *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
    - Exit from secondary perimeter to outside facilities:  
Health certificate w/disease specific endorsement

**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).

## Communicate the Plan

### I. Event Management

- Physical plant modification instructions
  - Barriers—designation of isolation facility & establishment of physical perimeter
  - Disinfection
  - Waste removal
  - Vermin control
- Personnel management
  - Requirements
  - Instructions
  - Notification of zoonotic risk

*All cases of equine diarrhea should be considered zoonotic until Salmonellosis has been ruled out.*

Link to *A Review of Equine Zoonotic Diseases: Risks in Veterinary Medicine* (J.S. Weese) <http://www.aaep.org/proceedings/02proceedings/910102000362.pdf>

- Outbreak updates

## **II. Veterinarians**

- Instructions— disease surveillance/testing/reporting
- [Biosecurity guidelines – link to document](#)
- Health requirements—entrance into/exit out of facility
- Outbreak updates

## **III. Horsemen**

- Disease information for horsemen/owners
- [Biosecurity guidelines – link to document](#)
- Human exposure/zoonotic risk management
- Instructions for caretakers
  - [Caretakers' Instructions](#)
  - 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 required to be aware of currently reportable diseases to the state veterinarian and abide by state regulations

Note: 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

### Tests

#### **Bacterial culture of feces**

Standard aerobic

Salmonella-specific—indicate on laboratory submission form

Anaerobic + Clostridial toxin testing

#### **Antibiotic susceptibility testing and serotyping of *Salmonella* spp. Isolate**

#### **PCR--Potomac Horse Fever**

#### **Serology**

## 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/](http://www.aavld.org/). Click on the Laboratory Directory: [www.aavld.com/aavld-3/lab.jsp](http://www.aavld.com/aavld-3/lab.jsp)

## Sample Collection

Aseptic technique (best) or free catch into urine cup

3-5 grams of feces or 3-5 cc of liquid feces (diarrhea)

or

2-3 rectal swabs if a quantity of feces is not obtainable

PCR— PHF submission of whole blood (lavender top) and/or feces

## Sample Handling

Submit fecal samples cooled (but not frozen); pack surrounded by freezer packs in insulated, leak-proof containers.

## Sample Transport

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

Use the correct submission form for the laboratory as provided by FAX or downloaded from the web.

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

If the complications of shipping are too great, put the sample in an appropriate cooled container, and have a courier drive it directly to the laboratory. Call first to find out where samples are received.

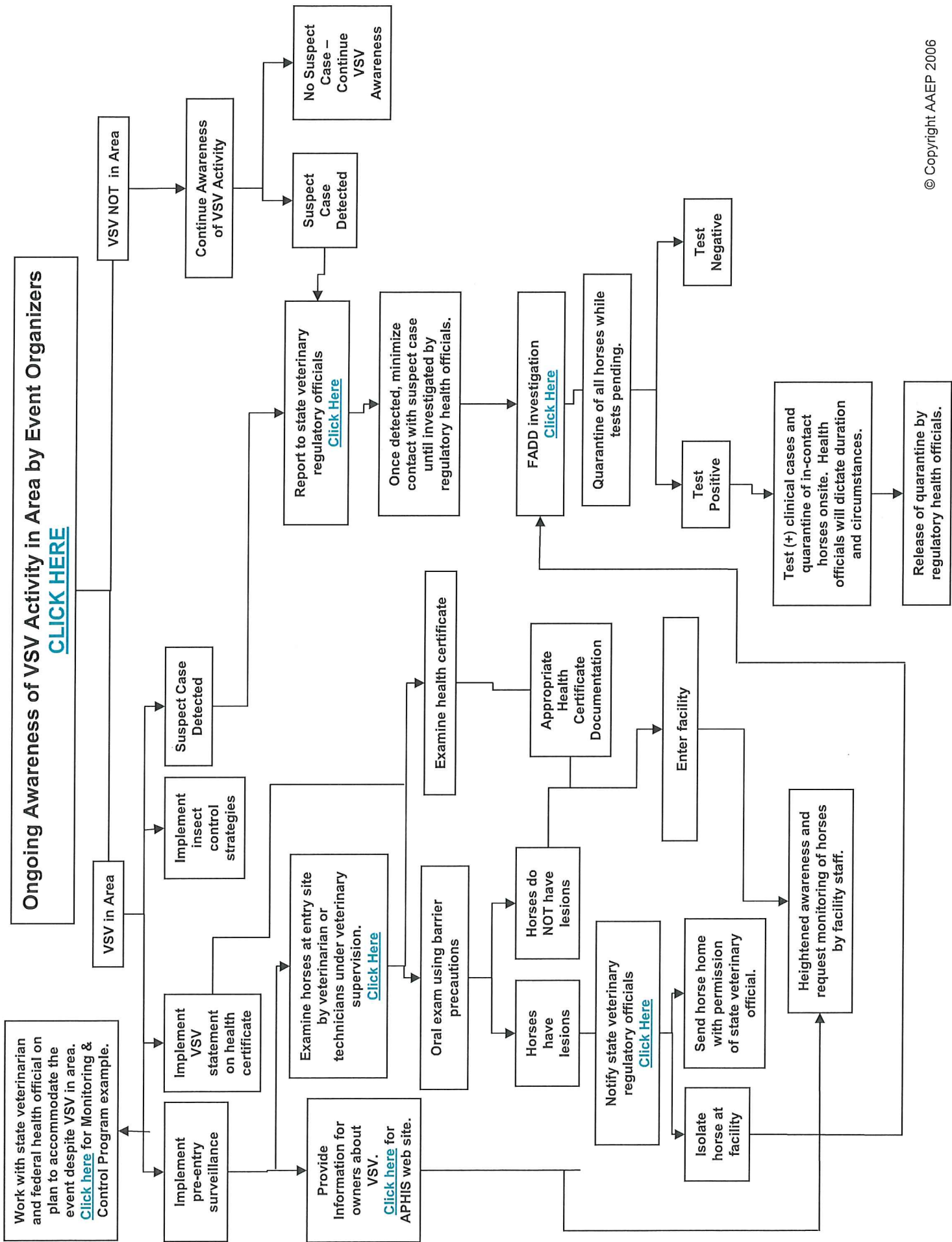
Safe shipping of samples:

**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 are as follows:

- 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)



## Vesicular Stomatitis

### Definition

Vesicular Stomatitis is a viral disease of horses, donkeys, mules, cattle, swine and New World camelids that occurs in the Western Hemisphere leading to vesicular lesions that rapidly progress to ulcerative stomatitis and other lesions such as coronitis and crusting dermatitis of the muzzle and nares.

### Clinical Signs

The primary clinical disease sign of vesicular stomatitis virus (VSV) infection in horses is ulcerative stomatitis with the tongue being most severely affected area in the oral cavity. Although the disease starts with vesicle formation it rapidly progresses to the ulcerative phase. The most common observation by owners is that the horse has excessive salivation and difficulty prehending and masticating feed. Coronitis, ulceration of the mucocutaneous junction of the lips and crusting of the muzzle and nares are less frequent clinical signs. Some infected horses can develop lesions on the udder and prepuce.

### Transmission

Vesicular stomatitis viruses are considered arboviruses because they use insect vectors as their primary means of transmission. Evidence of arthropod transmission of VSV is most compelling for sand flies and black flies. Other insect species may also be competent biologic and mechanical vectors of VSV. Propagation of VSV outbreaks may be enhanced by movement of infected horses and spread by direct contact between infected and uninfected mammalian hosts.

### Diagnostic Sampling, Testing and Handling

Laboratory testing is required to confirm VSV infection. There is a requirement in most states for the veterinary practitioner to report a case of vesicular or ulcerative disease to the [state/provincial animal health official](#) or [federal animal health officials](#).

The Report of a potential foreign animal disease (more recently termed a transboundary disease) by the private practitioner will prompt a standardized investigation by the animal health officials. The Foreign Animal Disease Diagnostician (FADD) will collect the appropriate samples and submit them for testing. There is no charge to the owner or private veterinarian for the FAD investigative procedures. Samples are tested at laboratories specializing in testing for FAD's either the [National Veterinary Services Laboratory](#) in Ames, IA or the [Plum Island Animal Disease Center](#) in New York, NY. Diagnosis is made through serologic testing and/or virus identification from samples of lesions.

### Post-mortem

If horses with VSV die or are euthanatized it would be due to secondary complications. Complications can result from the horse not drinking or eating due to painful oral lesions. Colic can occur due to ulceration in the esophagus and stomach. If nonsteroidal anti-inflammatory

drugs given to horses that are not drinking secondary renal failure can occur if hydration is not maintained by giving intravenous fluids.

*Practitioners performing necropsies in the field are encouraged to contact a veterinary diagnostic laboratory to which they plan to submit samples for further testing such as histopathology and pathogen identification in order to be certain they collect the appropriate samples and handle the samples in a manner that will optimize making a definitive diagnosis. For some situations such as neurologic cases submission of the entire carcass to the diagnostic laboratory for post-mortem examination is recommended due to the time and labor required to perform a complete exam and collection of samples from the equine CNS.*

### **Shedding Time of Organism Past Resolution of Clinical Signs**

The release of official quarantine of confirmed VS cases is based on resolution of lesions as the risk for virus transmission is considered minimal after lesions have healed.

### **Environmental Persistence**

Arboviruses generally use vertebrates as reservoirs for transmission by arthropods. Serologic evidence of exposure to VSV has been shown in many vertebrate species however the reservoir of VSV between outbreaks that occur periodically in the SW regions of the United States is unknown.

### **Specific Control and Treatment Measures**

#### Biosecurity Guidelines

Implementation of biosecurity practices to limit the spread of VSV is indicated.

- Wearing of disposable examination gloves when working with affected horses followed by hand washing is indicated.
- Eye protection is indicated when examining affected horses. VSV can cause flu like symptoms and stomatitis in humans with exposure potential from saliva from affected horses.
- Restriction of movement of affected horses and herd mates is important in control of spread of the virus and mandatory quarantine will be placed on confirmed affected premises by state or federal animal health officials.

#### Vaccination

There is currently no licensed commercially available vaccine in the United States for control of VSV.

#### Protection from Insect Exposure

Protecting horses from insect exposure during outbreaks of VS may reduce the risk of clinical disease. Options for reducing insect exposure include:

- Housing horses indoors during the evening
- Regular application of insect repellants to the horse including the inner surface of the ears (location black flies may feed).

### Requirements for New Arrivals to Facility or Event

During VS outbreaks, additional requirement for horses that have been in regions where disease has been recognized may reduce risk of introduction of VSV. These recommendations include:

- Certificate of veterinary inspection (CVI) with statement related to potential exposure to VSV
- Inspection of newly arriving horses including an oral examination to detect vesicles or ulcers. Wear disposable examination gloves and change between horses examined.

### Treatment

There is no specific treatment for VS in horses. The disease is typically short lived and self-limiting. Provide softened feed while oral ulcers are present. Horses should be assessed for dehydration and if it occurs then intravenous fluids maybe needed to resolve the problem. Rinsing of oral lesions with a mild antiseptic solution may reduce the risk of secondary bacterial infection but increases the risk of exposure of care givers to VSV and is usually not necessary except in severe cases. If treatment of oral lesions is pursued the care giver should use barrier precautions including eye protection to reduce the risk of exposure to VSV.

### **Release of Animals from Isolation**

Release of VS cases and their herd mates will be determined by state or federal animal health officials and is based on a specified number of days after resolution of lesions in those animals that had clinical signs.

### **Biosecurity Issues for Receiving Animals**

Once horses are released from quarantine no additional precautions should be needed to reduce the risk these horses pose for spread of VSV.

### **Zoonotic Potential**

There is evidence of human exposure to VSV resulting in illness through laboratory work and through working with affected animals. Based on findings from an outbreak of VS in Colorado a higher risk of seropositivity was observed in people who examined the oral cavity of infected animals and had open wounds on hands or arms and who examined horses rather than cattle. VS in humans is an acute, self-limiting infection with signs similar to influenza infection. Vesicular lesions have been documented to occur but rarely in humans exposed to VSV.

# Botulism

## Definition

Disease caused by toxins produced by *Clostridium botulinum* an anaerobic, spore forming bacteria ubiquitous in soil. Toxin proliferates under vegetative conditions.

Botulism is a rapid and progressive neurologic disease with high mortality (100% in untreated animals). Horses can ingest either bacteria which then proliferate in the gastrointestinal tract and secrete toxin (toxicoinfectious) or ingest pre-formed toxin.

## Clinical Signs

- Severe muscle weakness
- Flaccid paralysis with normal mentation
- Inability to swallow (foals will reflux milk from nostrils)
- Poor tail, tongue and eyelid tone
- Hypoventilation, respiratory arrest
- Paresis/inability to stand for extended periods
- Limb paralysis
- Progression to muscular weakness and recumbency

## Incubation

- 12-24 hours post-ingestion of toxins
- Toxicoinfectious and wound botulism have a variable onset based on proliferation of bacteria and toxin.
- Once elaborated, effects due to toxin occur within 24 hours as with ingestion of preformed toxin.

## Transmission

- Ingestion of pre-formed toxin in contaminated feed
- Toxicoinfectious—Shaker Foal Syndrome
  - Large amount of bacteria overgrows in gut then elaborates toxin
- Direct contact—wound contamination or via umbilicus in foals

## Diagnostic Testing

- Clinical signs
- Confirmatory testing is difficult and expensive:
  - Definitive diagnosis is achieved by identification of toxin in plasma, liver, or gastrointestinal tract.
  - Tentative diagnosis is based on identification of *C. botulinum* spores in gastrointestinal contents or wounds.
  - Animals that recover from the disease do have antibody present but commercially available testing is limited

## Shedding Time of Organism Past Resolution of Clinical Signs

There is no demonstrable shedding of *C. botulinum* once clinical signs occur, particularly if the source of infection is that of a wound or umbilicus.

**Environmental persistence (toxins)**

Toxins are susceptible to sunlight, 1-3 hours

**Specific Control Measures**Environmental management

Bleach is effective disinfectant (after thorough removal of organic material) for toxins and/or vegetative cells.

Clostridial spores are resistant to most environmental conditions and disinfectants.

Vaccination

Prevention of botulism in foals is approached through vaccination of broodmares. Understanding of regional variation in prevailing antigenic type is helpful in determining vaccine selection.

**Release of Animals from Isolation**

There are no isolation requirements for horses with this disease.

**Biosecurity Issues for Receiving Animals**

There are no biosecurity issues for housing and/or handling of these animals.

**Zoonotic Potential**

None.

## Clostridial Diarrhea in Adult Horses

### Definition

Clostridial diarrhea is caused by the gram positive, anaerobic, spore-forming bacteria *Clostridium spp.*. While the most common isolates are *C. perfringens* and *C. difficile*, multiple others, including *C. septicum*, *C. cadaveris* and *C. sordellii*, have also been associated with enterocolitis.

Clostridial bacteria are present in the environment and in manure.

In adult horses clostridial diarrhea has been associated with factors that may alter the balance of the intestinal flora such as the administration of antibiotics, but can occur in the absence of any identifiable risk factors.

### Clinical Signs

May occur as an individual case or cluster of horses with enterocolitis  
Diarrhea onset may be peracute (w/ rapid progression to death), acute, or gradual  
Diarrhea may be hemorrhagic, or dark and foul smelling  
Colic  
Fever, reduced appetite  
Septic shock  
Sudden death

### Incubation

Not known, as bacteria can be present in low numbers in normal equine intestinal tracts without any clinical signs.

Antibiotic-induced clostridial diarrhea usually occurs within the first week after initial administration.

### Transmission

While clostridial bacteria are considered to be part of normal equine GI flora, it is prudent to isolate any horse with acute diarrhea and take appropriate hygiene precautions.

### Risk Factors

Disruption of intestinal microflora is thought to induce overgrowth of intestinal toxigenic clostridia, resulting in diarrhea. Factors which have been associated with this include:

- Stress
- Hospitalization
- Surgery
- Administration of antimicrobials
- Sudden dietary changes
- Deworming of horses having a heavy parasite load

### Diagnostic Testing

Definitive diagnosis of clostridial colitis beyond *C. difficile* and enterotoxigenic *C. perfringens* is difficult.

Culture alone is not diagnostic as clostridia are often present in the intestinal tracts of normal animals.

*C. difficile* diarrhea diagnosis:

ELISA detection of toxins in fecal sample

Test should detect Toxins A and B, as opposed to A only

\*Consult laboratory for testing capabilities prior to submitting samples

Minimum sample—1 gram of feces or intestinal contents

*C. difficile* toxins are shed early in disease; a single sample is thought to be adequate.

Ship samples cooled on ice packs.

*C. difficile* toxins are stable in equine feces for many days if kept cool {Weese J Vet Diagn Invest 2001}.

*C. perfringens* diarrhea diagnosis:

Culture followed by PCR genotyping of the isolated *C. perfringens* to determine strain types can be useful in some circumstances, however the relevance of identification of most types in diarrheic feces remains unclear.

Type C strains seem to be more highly associated with disease and less common in healthy animals.

ELISA testing is available to detect presence of one *C. perfringens* toxin, called *C. perfringens* enterotoxin, in fecal samples.

Submit minimum 1 gram feces; ship on ice packs.

Some research laboratories perform testing for other lethal toxins of *C.*

*perfringens*—alpha, Beta 1 and Beta2 toxins—but these tests are rarely available at diagnostic laboratories.

Testing for other clostridial causes of enterocolitis is very difficult. Standard anaerobic cultures can be performed, but most clinical laboratories are not adequately equipped for anaerobic investigations. Interpretation of results remains difficult as it is often impossible to determine whether a *Clostridium* present in a single sample is causing disease or simply resident microflora.

## **Shedding Time of Organism Past Resolution of Clinical Signs**

Unknown; bacteria can be present in the gut flora and feces of normal horses.

## **Environmental Persistence**

Clostridial spores are impervious to environmental conditions but can be killed on very clean non-porous surfaces with bleach.

## **Specific Control Measures**

Consider all diarrheic horses as contagious until proven otherwise.

Routine isolation and disinfection guidelines should be followed, including proper disposal of manure.

Clostridial diarrhea can cause increased bacterial shedding and toxin levels in feces. Bedding and manure should never be spread on pastures.

**Release of Animals from Isolation**

After clinically normal.

**Biosecurity Issues for Receiving Animals**

Routine isolation measures to prevent any contagious disease spread.

**Zoonotic Potential**

*C. difficile* is a known cause of diarrhea in humans and has been documented to be a nosocomial infection in human health care facilities. Infection of humans from horses is not documented but it is advisable to take hygiene precautions when working with any diarrheic patients.

## Equine Herpesvirus 1 & 4 Related Diseases



### Definition

Equine herpesviruses are very common DNA viruses in horse populations worldwide. The two most significant are EHV-1, which causes respiratory disease, abortion, and neurologic disease; and EHV-4, which primarily causes respiratory disease and only occasionally can cause abortion or neurologic disease.

Equine herpes viral respiratory disease is usually caused by EHV-4 and is most commonly seen in weaned foals and yearlings, often in autumn and winter. Older horses are more likely than younger ones to transmit the virus without showing signs of infection. Although EHV-1 is the principal cause of outbreaks of viral abortion, some strains of EHV-4 have been associated with sporadic cases of the disease.

EHV-1 myeloencephalopathy (EHM) results from damage to the vasculature in the CNS. Certain strains of the virus are endotheliotropic; therefore, this results in vasculitis, thrombosis, and areas of infarction that lead later to foci of malacia in neurologic tissue. Herpesvirus myeloencephalopathy cases occur singly or as outbreaks affecting 20-50% of the at-risk population. Occurrences may or may not be preceded by a febrile episode or signs of respiratory disease. [See ACVIM Consensus Statement.](#)

### Clinical Signs

#### Fever

Fever is often biphasic and can be transient. The initial febrile phase precedes infection of the upper respiratory tract. The second febrile phase (6-7 days) often precedes a systemic viremia. Fever may go undetected and may be the only clinical sign noted in an infected horse. Temperature monitoring twice a day is recommended.

#### Respiratory disease

- Fever (A horse whose body temperature is >101.0 or 1.5 degrees greater than the horse's normal body temperature is considered febrile.)
- Coughing
- Nasal discharge
- Variable enlargement of the mandibular and/or retropharyngeal lymph nodes
- Lethargy, anorexia
- Conjunctivitis
- Ocular disease including uveitis and keratitis
- Neonatal foals infected in utero are usually abnormal from birth and exhibit any combination or all of the following:
  - Fever
  - Lethargy
  - Weakness
  - Jaundice
  - Respiratory distress/stridor/pneumonia
  - CNS signs (occasionally)
  - Death commonly occurs within 3 days.
- Older foals: nasal discharge is the most common sign of disease.

## Abortion

Most often, no warning signs of impending abortion in the mare. Typically occurs in late pregnancy (7+ months); very occasionally as early as 4 months.

## Neurologic disease:

- Incoordination of the hind (and occasionally fore) limbs
- Ataxia or wobbly gait
- Urine retention/dribbling
- Bladder atony
- Recumbency with inability to rise
- Neurologic signs are often preceded by fever and/or respiratory signs

## **Incubation Period**

After exposure by any route, incubation period may be as short as 24 hours but is typically 4-6 days, or longer.

*Note: EHV-1 abortion can occur from two weeks to several months following exposure to the virus with mares showing no clinical signs.*

## **Risk Factors**

- Evidence of transmission of EHV-1 virus
- Strain of EHV-1 virus detected
- Number of horses potentially exposed (Areas of high commingling of horses such as racetracks, hospitals, show grounds, etc...)
- Immune status of exposed horses, i.e. hospital or geriatric, horse rescue (Stress or immunosuppression: transport, hospitalization, training, showing, weaning, high doses of steroids)
- Testing results of exposed and clinically affected horses
- Movement of horses once released from restrictions/isolation

## **Transmission**

Respiratory transmission (most common route of exposure)

Inhalation of droplets from coughing and snorting. (Note: EHV is not believed to spread by this route as efficiently as equine influenza virus.)

Mares which have aborted, or whose foals have died, can transmit infection via the respiratory route.

Shedding by the respiratory route typically lasts for 7-10 days, but can be much longer. Therefore, based on a thorough risk analysis of the particular outbreak or case, a period of 14 to 28 days after resolution of clinical signs may be necessary before release from movement restrictions/isolation. EHV-1 testing of horses considered exposed or infected provides increased confidence in the release of restrictions/isolation period prior to 28 days.

### Direction transmission

Aborted fetuses, fetal membranes and/or fluids are significant sources of the virus.

Infected foals are highly contagious and can transmit infection to other horses via the respiratory route through shedding virus into the environment.

### Indirect transmission

Virus can be viable for several weeks in the environment once it has been shed by the horse.

Virus contaminated fomites are a significant factor in EHV spread.

## Diagnostic Sampling, Testing and Handling

### EHV 1 and 4

<u>Sample</u>	<u>Test</u>	<u>Shipping</u>	<u>Handling</u>
Nasopharyngeal Swab  and  EDTA or citrated blood  Both blood and NP swab should be tested together	EHV 1 or 4 PCR; Viral Isolation	PCR testing send swab in plain red top tube; for viral isolation place swab in viral transport media or red top tube with a few drops of sterile saline	Chilled overnight
Sera	EHV 1 and 4 Serum neutralization (SN)	Leak proof container	Chilled overnight
Sera	CF	Not available in US	Chilled overnight
Sera	ELISA* Differentiation of EHV 1 from 4	Leak proof container	Chilled overnight
	* specific antibodies directed against viral glycoprotein gG ( <a href="#">Svanovir™</a> )		

The [ACVIM consensus statement](#) indicates that real time PCR (RT-PCR) is more sensitive than conventional PCR. RT PCR testing is recommended over conventional PCR testing. Note: RT PCR will allow for more sensitive detection, greater specificity.

Serology - Serological diagnosis using the viral neutralization test (synonym: serum neutralization [SN] test) cannot distinguish between EHV-1 and EHV-4 infection. Nevertheless, in combination with specific clinical signs, a four-fold or greater rise in antibody titer is confirmatory of the infection. When a single virus neutralization titer is very high (typically 1:1024 to 1:2048 or greater) this is likely to be the result of recent infection rather than vaccination.

The complement fixation (CF) tests have been shown to be useful in the diagnosis of recent infection; however, commercial testing is currently unavailable in N. America.

A commercial ELISA test kit, suitable for use in practice, is available for detection and differentiation of EHV-1 and EHV-4 specific antibodies directed against viral glycoprotein gG ([Svanovir™](#)).

Contact your diagnostic laboratory for specific instructions regarding sample procurement and further information regarding sampling. Many diagnostic laboratories sell viral transport media and other products. Other sources are available from veterinary distributors.

#### [Nasal Swab/Transport Media Resource Option](#)

(Note: This is just one resource and is not specifically a recommended or endorsed supplier or product of the AAEP.)

### **Nasopharyngeal or Nasal Swab Collection procedure for EHV 1/4 and related diseases**

#### Supplies Needed

- Dacron Swabs with plastic sticks 5-7 inches ( often supplied with bacterial transport medias)
- Plain red top tube (blood tube) or viral transport media
- Few milliliters sterile saline
- Clean exam gloves
- Disinfectant material or wipe

#### Procedure

1. Horse should be restrained. Pass the swab(s) along the left/ right ventral meatus being careful to avoid the false nostril, until you are in the horse's nasal passage. Rotate the swab to increase the collection of respiratory secretions. Two swabs can be done at the same time if more than one sample is needed by the laboratory. Some labs request one sample for PCR and one for viral isolation.
2. Place swab(s) into plain red top tube with 1-2 drops of sterile saline for PCR/viral isolation. **DO NOT PLACE PCR/viral isolation SAMPLES IN BACTERIAL TRANSPORT MEDIA.**
3. If sampling more than one horse, fresh gloves should be used for each animal.

4. The outside of tubes should be wiped down with disinfectant to prevent contamination of other samples or gloves of handlers.
5. Consider all waste materials to be infectious including gloves and swabs, etc.
6. Any restraining equipment such as twitches, nose chains, etc. must be disinfected between use.

### **Post-mortem**

**EHM:** Fatal outcome in horses infected with EHV-1 are most often associated with the neurologic form of the disease, EHM. Definitive diagnosis of EHM in an individual horse is only possible after histological and immunohistochemical examination of CNS tissues in many cases.

Examination of fresh CNS tissue by PCR can also be attempted. This requires dissection of the entire spinal cord, followed by both gross and extensive histological examination of each part of the spinal cord.

This procedure is both time- and labor-intensive, and is typically only practical at a properly equipped necropsy laboratory, typically at a state diagnostic laboratory, or at a school of veterinary medicine.

In the USA, the necropsy facility should be accredited by the [American Association of Veterinary Diagnosticians](#). A full list of accredited laboratories is maintained on their website.

The complete carcass should be presented for post-mortem examination as soon as possible after death.

**EHV abortion:** When EHV results in abortion the fetus is usually expelled while still in the placenta and within the amniotic membrane. Almost all abortions occur in the last 4 months of pregnancy. Both the placental and fetal tissues should be submitted for necropsy and specific testing to detect EHV. Biosecurity precautions are indicated when handling the placenta and fetus as both can contain high levels of infective virus. Detailed instructions for veterinarians on how to collect and submit aborted equine fetal and other diagnostic samples from the mare are available at the [Animal Health Diagnostic Center at Cornell University](#).

### **Shedding Time of Virus Following Resolution of Clinical Signs**

Possibly up to a week, but may be longer in exceptional cases.

Recovered horses typically develop latent infections and are capable of shedding virus following reactivation (with or without signs of clinical disease) particularly under conditions of stress, for the remainder of their lives.

Horses with residual stable neurological signs are not considered when determining the countdown to release from isolation.

### **Environmental Persistence**

Environmental transmission plays a minor role in the maintenance of virus in the horse population since environmental persistence of EHV-1 is short, estimated to be no more than 35 days under ideal conditions and probably less than 7 days in most practical field situations.

## Specific Control Measures

### Biosecurity

Please view and follow the [AAEP Biosecurity Guidelines](#).

Clinically normal horses housed within the primary perimeter may be permitted segregated exercise periods outside the perimeter. Precautions should be taken, and may include:

- Exercise scheduled *after* general population's exercise period to avoid potential virus transfer to unaffected horses/barns by exercise riders.
- Access to starting gate or similar equipment denied.
- Restricted use of ponies/outriders' horses – horses housed within the primary perimeter may only be escorted by horses housed within the same facility.
- Direct horse-to-horse contact is to be avoided.
- Prompt post-contact use of alcohol hand sanitizer by individuals having contact with horses during exercise.

### Vaccination

Booster vaccination of healthy animals in both primary and secondary contagion control perimeters may have some value, and is not known to lead to complications. If animals are unvaccinated prior to an outbreak there is unlikely to be time to administer an effective vaccination series during the risk period based on currently available EHV-1 vaccines. Do not vaccinate clinically ill animals. Please see [AAEP Vaccination Guidelines](#).

### Release of Animals from Isolation

Maintain isolation procedures for 14-28 days after last clinical signs are detected, basing the release date on risk analysis. A shorter quarantine period may be justified, such as 21 days, if during this time:

1. No horse had a fever (temperature taken at least one time every 24 hour period and without any treatment of non-steroidal anti-inflammatory drug)
2. No horse had an abortion
3. No new cases of neurologic disease (Note: Neurological clinical signs are considered to be resolved when they stabilize, i.e. residual neurological signs are not considered in determining a day 0 for countdown of release of restrictions/isolation.)
4. All exposed horses have been tested and have given a negative result based on testing nasal swabs for EHV-1 by real-time PCR. EHV-1 testing of horses considered exposed or infected would allow for increased confidence in the release of restrictions/isolation prior the 28 day time period. There should be compliance with requirements by state animal health officials for duration of quarantine and testing.

## Biosecurity Issues for Receiving Animals

Horses having been housed within primary perimeter:  
Isolate from general population for 28 days

Horses having been housed within secondary biosecurity perimeter:

After having determined its level of risk-aversion, the recipient facility may consider the following:

1. Vaccination requirements for entrance into facility
2. Health certificate specifications
3. Test findings (a negative PCR result on a nasal swab)

Update vaccination for animals at recipient facility before arrival of potentially infected/exposed animal.

**Zoonotic Potential**

None known

# Equine Viral Arteritis

## Definition

Equine viral arteritis (EVA) is a contagious viral disease of horses worldwide. The disease is frequently confused with other conditions that produce clinically similar conditions. Documented outbreaks have been infrequent, but this may be related to lack of recognition. The portion of the name arteritis comes from the characteristic vascular lesion that is produced.

## Clinical Signs

Variable (similar to any infectious agent that causes vasculitis) but most commonly:

Fever, depression, anorexia

Edema

Limbs, ventrum, periorbital region, scrotum/preputia (male), mammary (female)

[Urticaria - Link to EVA photos](#)

Conjunctivitis

Rhinitis

Abortion—associated with ‘abortion storms’

Leukopenia

Mortality is rare in adult horses.

**Note:** The majority of acute EVA infections are subclinical or inapparent.

## Transmission

Aerosol transmission via respiratory secretions of acutely infected horses predominates.

Venereal transmission

Stallions can become carriers and maintain the virus in the horse population.

Cooled or frozen semen can be infectious.

Indirect transmission (fomites, urine, feces, artificial insemination, and vaginal secretions) has been reported

Congenital infection in foals has been reported.

## Diagnostic Testing

Diagnosis based on clinical signs is difficult due to the wide array of clinical signs and frequency of unapparent/mild signs.

Diagnosis is made by virus isolation and/or paired serology.

Virus isolation sampling can include whole blood (EDTA or heparin), nasopharyngeal swabs, conjunctival swabs, fetal or placental tissues/fluids.

Virus isolation sampling should be initiated promptly once clinical signs manifest. EVA is fragile at room temperatures, but stable when frozen. Samples should be frozen and shipped with freezer packs.

Paired (acute and convalescent) sera should include a time interval of 3-4 weeks. Previous vaccination history should be considered when interpreting titers. Vaccinated individuals may have an active serologic response or a rapid rise in titer in response to exposure.

## Shedding Time of Organism Past Resolution of Clinical Signs

Carrier stallions continue to shed through semen, but not through the respiratory tract.

Only stallions develop into the carrier state.

## **Environmental Persistence**

The virus is heat sensitive but is able to persist at freezing temperatures.

## **Specific Control and Treatment Measures**

### Biosecurity Guidelines

Clinically normal horses housed within the primary perimeter may be permitted segregated exercise periods outside the perimeter. Precautions should be taken, and may include:

- Exercise scheduled *after* general population's exercise period to avoid potential virus transfer to unaffected horses/barns by exercise riders.
- Access to starting gate or similar equipment denied
- Restricted use of ponies/out-riders' horses—horses housed within the primary perimeter may only be escorted by horses housed within the same facility.
- Direct horse-to-horse contact is to be avoided.
- Prompt post-contact use of hand sanitizer by individuals having contact with horses during exercise.

### Vaccination

The vaccine has been used successfully to curtail the spread of EVA in large outbreaks of the disease. In the face of an outbreak, vaccination of exposed but clinically normal horses can be used as a part of a control program.

Primary vaccination provides protection from clinical disease for 1-3 years. Vaccination does not prevent re-infection or potential replication of challenge virus.

Note: Vaccinated horses may become viremic and transmit disease while remaining clinically normal.

Revaccination results in enhanced serologic response.

It is recommended that at-risk stallions be re-vaccinated annually.

Stallions are screened serologically before primary vaccination. Implications regarding breeding shed access and export must be considered when vaccinating at-risk horses.

Currently it is not possible to differentiate vaccinate from natural infection via serology.

'Pony' horses/outriders' horses/catch horses (or those with close contact to multiple horses) should be vaccinated or withdrawn from use until vaccinations are administered.

## **Release of Animals from Isolation**

Release of animals from the primary perimeter may be considered 3-4 weeks after the resolution of clinical signs within the primary perimeter. Animals can be released if virus isolation (nasal/pharyngeal swab and blood) are negative. After all animals have been released (3-4 weeks post clinical signs/ negative virus isolation) from the primary perimeter a thorough disinfection of the area should be attempted.

## **Biosecurity Issues for Receiving Animals**

For horses having been housed within the primary perimeter:

Certificate of Veterinary Inspection w/ affidavit indicating that within the previous 21 days the horse has not demonstrated signs of EVA, has not been exposed to nor housed with horses that demonstrated signs of, or were suspected or confirmed as being infected with EVA.

For other horses:

Require health certificate w/disease specific disclaimer and proof of vaccination.

Breeding farms:

Mares or fillies shipping from a premise of exposure should follow the requirements as listed for exposed and unexposed individuals as above.

Colts and stallions should also follow these restrictions (in addition to any State veterinary restrictions) if these animals are to enter the breeding population.

### **Zoonotic Potential**

None known.

# Equine Influenza Virus

## Definition

Equine influenza virus is a RNA virus that occurs sporadically in epidemic form in horse populations worldwide (except in Iceland, Australia or New Zealand).

## Clinical Signs

Fever  
Coughing (sometimes paroxysmal)  
Mucopurulent nasal discharge  
Distal limb edema may occur  
Cardiac myopathy has been reported in affected animals

**Incubation:** Potentially as short as 24 hours and up to 3 days.

## Transmission

Aerosol transmission (most common)  
Inhalation of droplets from coughing and snorting (may be able to spread as far as 50 yards by this route).

Respiratory shedding typically lasts for 7-10 days in naïve animals; much shorter shedding periods occur for partially immune horses.

Indirect transmission can occur.

## Diagnostic Testing

Virus isolation  
PCR  
Immunoassay—stall-side kit  
Serology—paired titers may be of use

## Shedding Time of Organism Past Resolution of Clinical Signs

Probable for 7 days, possible up to 10 days.

## Environmental Persistence

Virus can remain viable for up to 2 days on fomites.  
In water, virus viability has been reported up to 3 days.

## Specific Control Measures

### [Biosecurity Guidelines](#)

#### Vaccination

Booster vaccination of healthy animals in primary and secondary contagion control perimeter is likely to be of value, and is not known to lead to complications.

If animals are unvaccinated prior to the outbreak, the use of a modified live intranasal vaccine is recommended as this can achieve protection within 5 days of primary administration.

Release of animals from isolation:  
Maintain isolation procedures (primary perimeter) for 21 days after resolution of last suspected new infection.

### **Biosecurity Issues for Receiving Animals**

For horses having been housed within primary perimeter:  
Isolate from the general population for 14 days

For other horses:  
Vaccination requirements may be considered when disease risk is elevated.  
Certificate of Veterinary Inspection specifications—disclaimer regarding disease exposure within a specified interval.

### **Zoonotic Potential**

None known.



## Pigeon Fever (*Corynebacterium pseudotuberculosis* infection)

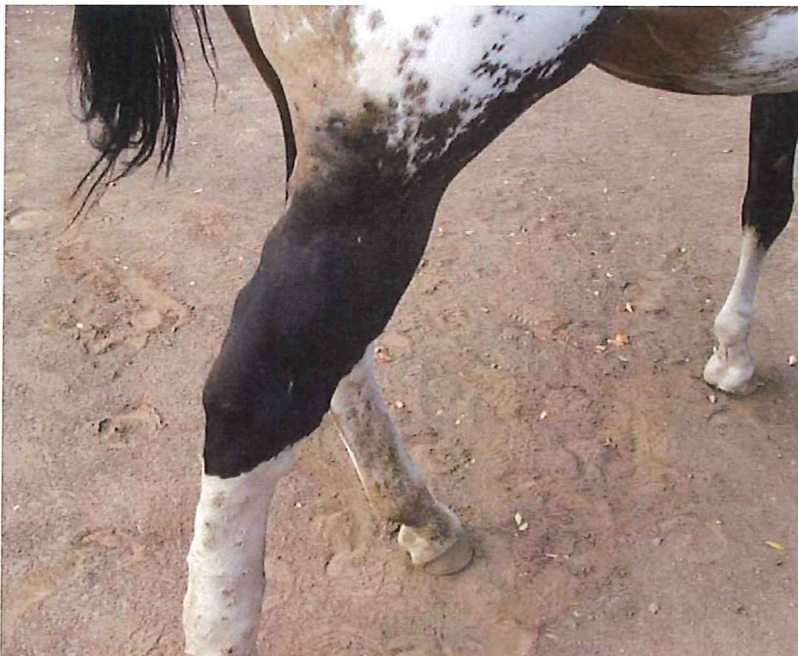
### Definition

*Corynebacterium pseudotuberculosis* is a gram-positive bacteria with worldwide distribution. In North America, cases have been reported throughout the United States. Infection has been reported in equids, sheep, goats, cattle, buffalo, camelids, and rarely humans. Biotypes isolated from small ruminants and camelids are nitrate negative, while those from horses are nitrate positive. Natural cross-species transmission does not occur between sheep and horses, however cattle can have infection from either biotype.

### Clinical Signs

Three forms have been described in horses: ulcerative lymphangitis or limb infection, external abscesses and internal infection. Ulcerative lymphangitis and internal infection must be treated more aggressively with antimicrobial therapy, while use of antimicrobials for external abscesses is often unnecessary.

*Ulcerative lymphangitis* is the least common form seen in North America, although this form of disease has been reported worldwide. *Ulcerative lymphangitis* manifests as a severe limb swelling and cellulitis, with multiple draining tracts following lymphatics. Most commonly one or both hind limbs are affected. Horses often develop a severe lameness, fever, lethargy and anorexia. Aggressive medical therapy (antimicrobial and anti-inflammatory) is necessary or the disease often becomes chronic, resulting in limb edema, prolonged or recurrent infection, lameness, weakness, and weight loss.



**Ulcerative lymphangitis** (Photo by Sharon Spier, DVM, Ph.D, University of California, Davis)

*External Abscesses* are the most common manifestation, and may occur anywhere on the body, but most frequently develop in the pectoral region (swelling resembles a pigeon's breast) and along the ventral midline of the abdomen. Abscesses contain tan, odor-free purulent exudate and are usually well encapsulated. Additional sites for abscess formation are the prepuce, mammary gland, triceps, axilla, limbs, and head. Septic joints and osteomyelitis have been reported. Horses may have a single or multiple abscesses involving different regions of the body. Horses with external abscesses do not usually develop signs of systemic illness, however one-quarter will develop fever. If signs of systemic illness are present, further diagnostics to rule out internal infection are warranted, and antimicrobial therapy should be considered. While there is considerable variation in severity among horses, most straight – forward cases can be treated with lancing and draining the abscesses when mature. The case fatality for horses with external abscesses is very low (0.8%).



**Typical pectoral abscess with flies attracted to exudates**  
(Photo by Sharon Spier, DVM, Ph.D, University of California, Davis)

*Internal infection* occurs in approximately 8% of affected horses, which is associated with a high case fatality rate (30 to 40%). Diagnosis can be challenging, and long-term antimicrobial therapy is imperative for successful outcome. In a retrospective study, the organs most commonly involved were liver, kidney, spleen and lungs. Abortion due to placentitis or fetal infection has been reported.

## Transmission

The portal of entry of this soil-dwelling organism is thought to be through abrasions or wounds in the skin or mucous membranes. Many insects have been incriminated as vectors for the transmission of the disease to horses, and studies have shown that *Haematobia irritans*, *Musca domestica*, *Stomoxys calcitrans* can act as mechanical vectors of this disease. The regional location of abscesses suggests that ventral midline dermatitis is a predisposing cause of infection. Temporal and special analysis indicated an incubation period of 3 to 4 weeks. Within a geographic area, the disease appeared to be transmitted between 7 and 56 days throughout a 4.3 to 6.5 km distance, strongly suggesting that the disease could be transmitted through horse-to-horse contact or from infected to susceptible horses via insects, other vectors, or contaminated soil. The organism has been shown to survive for up to 2 months in hay and shavings, and more than 8 months in soil samples at environmental temperatures. The incidence of disease fluctuates considerably from year to year presumably due to herd immunity and environmental factors such as rainfall and temperature. Disease incidence is seasonal, with highest number of cases occurring during the dry months of the year, which is summer and fall in the Southwestern US, although cases may be seen all year. Horses with internal infection are more frequently seen one to two months following the peak number of cases with external abscesses.

## Diagnostic Sampling, Testing and Handling

Bacterial culture of aspirates or exudate is used to confirm diagnosis and the organism survives for prolonged periods. *Corynebacterium pseudotuberculosis* grows well at 36°C on blood agar in 24 to 48 hours, and it forms small, pinpoint in diameter, whitish, opaque colonies that are surrounded by a weak zone of hemolysis. Biotypes isolated from small ruminants and camelids are nitrate negative, while those from horses are nitrate positive. *Corynebacterium pseudotuberculosis* produces various extracellular exotoxins, which play a role in virulence; the most studied is phospholipase D (PLD). The bacterial phospholipase D is similar to the PLD of the brown recluse spider, which explains the presence of pain and edema at the site of infection. The synergistic activity of *C. pseudotuberculosis* exotoxins with the exotoxins of *Rhodococcus equi* in lysing red blood cells in agar forms the basis for the synergistic hemolysis inhibition (SHI) test. The SHI test is used to detect IgG antibody to *C. pseudotuberculosis* in horses with internal infections where external abscesses are not present.

Clinical pathologic abnormalities that may be observed include anemia of chronic disease, leukocytosis with neutrophilia, hyperfibrinogenemia, and hyperproteinemia. These hematological parameters can occur with either internal or external abscesses but are more consistently observed with internal abscesses.

A diagnosis of *internal infection* is based on clinical signs, clinicopathologic data, serology, diagnostic imaging and bacterial culture. The most common clinical signs are concurrent external abscesses, decreased appetite, fever, lethargy, weight loss, and signs of respiratory disease or abdominal pain. Other signs observed in horses with internal abscesses include ventral edema, ventral dermatitis, ataxia, hematuria (due to renal abscesses), and uncommonly, abortion.

*Serologic testing* using the Synergistic Hemolysis Inhibition (SHI) test can be useful in aiding the diagnosis of internal abscesses and is available through the [California Animal Health and Food Safety Laboratory System](#) in Davis, California, and the [Colorado State University Veterinary Diagnostic Laboratories](#) in Fort Collins, Colorado. Serology is generally not helpful for diagnosis of external abscesses and may be negative early in the course of disease and even the time of abscess drainage. Positive SHI titers must be interpreted carefully and combined with clinical signs to distinguish active infection from exposure or convalescence. Both published and unpublished data from the University of California suggests a reciprocal titer of  $\geq 256$  is indicative of active infection. Horses with internal abscesses generally have SHI titers  $\geq 512$ . Titers  $\leq 16$  are considered negative, while titers between 16 and 128 are considered suspicious or indicative of exposure. These are rough guidelines, however, as there is considerable overlap in results from horses with active disease, exposure and recovery from infection. *The SHI test is most accurate for diagnosis of internal infection in **the absence** of external abscesses. The SHI test should not be used alone to diagnose internal infection without other supportive diagnostics.*

Abdominal ultrasonography is the most useful tool for identifying affected internal organs and also for revealing the nature and extent of involvement. Abdominal ultrasonography also facilitated transcutaneous liver and kidney biopsy procedures and aspiration of abscess fluid for definitive diagnosis. Ultrasonography should be used in conjunction with hematologic and serum biochemical analyses to monitor response to treatment and may be the only available modality to monitor horses in which there is no clinicopathologic evidence of organ disease.

### **Environmental Persistence**

The organism has been shown to survive for up to 2 months in hay and shavings, and more than 8 months in soil samples at environmental temperatures. In experimental studies, the presence of manure favored survival and replication of bacteria in soil.

### **Specific Control and Treatment Measures**

#### Biosecurity Measures

Implementation of biosecurity practices to limit the spread of *Corynebacterium pseudotuberculosis* are aimed at reducing environmental contamination and spread via insects or fomites. The bacterium is endemic in many regions of the world and survives for months in soil, particularly when contaminated with manure.

- Wearing of disposable examination gloves when working with affected horses followed by hand washing is indicated.
- Isolation of affected horses from naive herd mates
- Protecting horses from insect exposure by regular application of insect repellants to the horse including the ventral midline (prevention of ventral midline dermatitis).
- Meticulous wound care (topical fly repellants, antimicrobial ointments and bandages) to prevent infection from a contaminated environment

### Vaccination

There is currently no licensed commercially available vaccine in the United States for control of *Corynebacterium pseudotuberculosis* in horses. Use of autogenous bacterin - toxoids designed for horses demonstrated increased SHI titers following 2 injections, however the protection remains to be established. A commercial bacterin - toxoid is clearly needed to protect horses as the disease becomes endemic in more geographic regions.

### Treatment

The treatment regimen for external abscesses must be individualized for each horse depending on the severity of disease, including the presence of systemic illness such as fever or anorexia, the extent of soft tissue inflammation, the maturity of the abscess and the ability to successfully establish drainage of purulent exudate. Establishing drainage is the most important treatment and ultimately leads to faster resolution and return to athletic performance. The proximity of the fibrous abscess capsule to the skin varies, often being <1 cm deep for ventral midline abscesses, to greater than 10 cm deep underlying muscle for some pectoral, axillary, triceps or inguinal abscesses. Aspiration and drainage of superficial abscesses is easily performed, however the use of diagnostic ultrasound is helpful for localization of deeper abscesses and to judge maturity of the abscess and proximity to the skin. The abscess contents and lavage solutions such as saline with or without antiseptic should be retrieved and disposed of to prevent further contamination of the immediate environment.

### ***Antimicrobial therapy***

Antimicrobials are indicated for horses with ulcerative lymphangitis and for horses with internal abscesses. The use of antimicrobials for external abscesses is not necessary in many horses and may prolong the time to resolution. Antimicrobial therapy may be justified when signs of systemic illness are present, such as fever, depression and anorexia, or when extensive cellulitis or lameness is present. Horses with deep intramuscular abscesses that are lanced and draining through healthy tissue may also benefit from antimicrobial therapy.

*Corynebacterium pseudotuberculosis* is susceptible *in vitro* to many antimicrobials commonly used in horses, including penicillin G, macrolides, tetracyclines, cephalosporins, chloramphenicol, fluoroquinolones and rifampin. Several factors should be considered when choosing an antimicrobial. The intracellular location of the organism, the presence of exudates and a thick abscess capsule, and the duration of therapy are important as are the cost of the drug and the convenience of administration. Despite *in vitro* susceptibility, the nature of the bacteria and the copious exudate render certain antimicrobials ineffective for some cases. Trimethoprim-sulfa (5 mg/kg based on the trimethoprim fraction, twice daily orally) or procaine penicillin (20,000 U/kg twice daily intramuscularly) are effective for external abscesses especially on the ventral midline. Rifampin (2.5-5 mg/kg twice daily orally) in combination with ceftiofur (2.5 – 5 mg/kg twice daily intravenously or intramuscularly) appears highly effective for treatment of internal abscesses. Internal abscesses have also responded favorably to enrofloxacin (7.5 mg/kg once daily orally). The average duration of antimicrobial

therapy for internal infection is 4-6 weeks, and is best determined by repeat abdominal ultrasound and clinicopathologic results.

Horses with *ulcerative lymphangitis* or cellulitis should be treated early and aggressively with antimicrobials or residual lameness or limb swelling may occur. Typically intravenous antimicrobials (ceftiofur or penicillin G) alone or in combination with rifampin (orally) are used until lameness and swelling improves, and then therapy with orally administered antimicrobials such as trimethoprim sulfamethoxazole or rifampin are continued to prevent relapse. The time to resolution reported in one study was approximately 35 days. Physical therapy, including hydrotherapy, hand walking, and leg wraps, as well as NSAIDs are recommended.

### **Biosecurity Management for Receipt of Animals**

Once horses are recovered and there is no drainage from abscesses no precautions should be needed to reduce the risk these horses pose for spread of infection. There is no practical way at this time for eliminating the bacteria from soil.

### **Zoonotic Potential**

There exist few reports of human illness through working with infected sheep, mostly in Australian sheep shearers who had open wounds on their hands and developed axillary lymphadenitis. One veterinary student from California developed pneumonia following exposure to an infected horse, presumably from inhalation of the bacteria from a contaminated environment.

## Potomac Horse Fever (equine monocytic ehrlichiosis, equine ehrlichial colitis, or acute equine diarrhea syndrome)

### Definition

Potomac Horse Fever (PHF) is caused by the rickettsial organism *Neorickettsia risticii* (previously known as *Ehrlichia risticii*). The disease can affect any age, breed or sex of horse. PHF cases usually occur in summer and fall.

### Clinical Signs

Highly variable, including:

- Diarrhea (mild to severe)
- Fever --up to 107°F (41.6° C), depression, anorexia, lethargy
- Laminitis
- Mild colic
- Decreased abdominal sounds
- Edema of limbs and ventral body, prepuce of males
- Abortion (by transplacental transmission)

Note: Concurrent infections with *Salmonella* have been documented.

### Incubation

Approximately 1-3 weeks

### Transmission

Oral ingestion of trematodes present in aquatic insects (typically associated with horses housed on pastures around creeks and rivers; PHF can occur in animals housed in racetrack stalls as well).

Whole blood transfusion from an infected donor

Transplacental

Other modes of transmission are under investigation

Note: Affected horses are not considered to be contagious by natural contact with other horses.

### Diagnostic Testing

PCR (buffy coat of blood sample, and fecal sample)

IFA titers: (Results interpretation must be made in conjunction with laboratory personnel and PHF vaccination history)

### Shedding Time of Organism Past Resolution of Clinical Signs

Confirmed PHF cases are not considered contagious.

### Environmental Persistence

The organism is within aquatic insects and not known to be free in the environment.

### **Specific Control and Treatment Measures**

Consider all diarrheic horses as contagious until proven otherwise.  
Routine isolation and disinfection guidelines should be followed, including proper disposal of manure.

### **Release of Animals from Isolation**

If PHF is the only cause of the illness, it is not considered a contagious disease, although any diarrheic horse should be isolated from other sick animals until normal feces is produced.

### **Biosecurity Issues for Receiving Animals**

None

### **Zoonotic Potential**

None known.

## Rabies (*Rhabdovirus*)



### Definition

Rabies is a fatal viral disease of mammals. Its occurrence is relatively infrequent in horses (as compared to other infectious neurologic diseases). The [Centers for Disease Control and Prevention](#) report that equids account for less than 1% of all rabies cases in the USA; the total number of equine cases has historically ranged from 42-82 annually.

### Clinical Signs

- Highly variable
- Disease is rapidly progressive with the interval from onset of clinical signs to death of approximately 5-7 days. The interval is usually shorter in unvaccinated horses.
- Insidious onset is the hallmark of equine rabies with frequently reported initial clinical signs of lameness, colic, dysuria, priapism in addition to overt signs of neurologic disease.
- Physical signs may include:
  - Fever
  - Anorexia
  - Blindness
  - Dysphagia
  - Hyperesthesia – manifest as self-mutilation
  - Muscle twitching
  - Lameness
  - Paresis and/or ataxia
  - Incontinence
  - Paralysis – ascending
  - Sudden death
- Behavioral signs:
  - Dumb form: depression/stupor
  - Furious form: mania – these horses are extremely dangerous

**NOTE: Rabies should be included as a differential in all neurologic patients.**

### Incubation Period

Typically 2-6 weeks, although longer incubation periods have been reported.

### Risk Factors

- Unvaccinated horses
- 24-hour access to pasture
- Resident in endemic area

## Transmission

Exposure occurs primarily through a bite wound from an infected animal.  
Aerosolization of nerve or central nervous system (CNS) tissues during necropsy.

## Diagnostic Sampling, Testing and Handling

Correct sampling and testing is imperative for a definitive diagnosis in rabies.

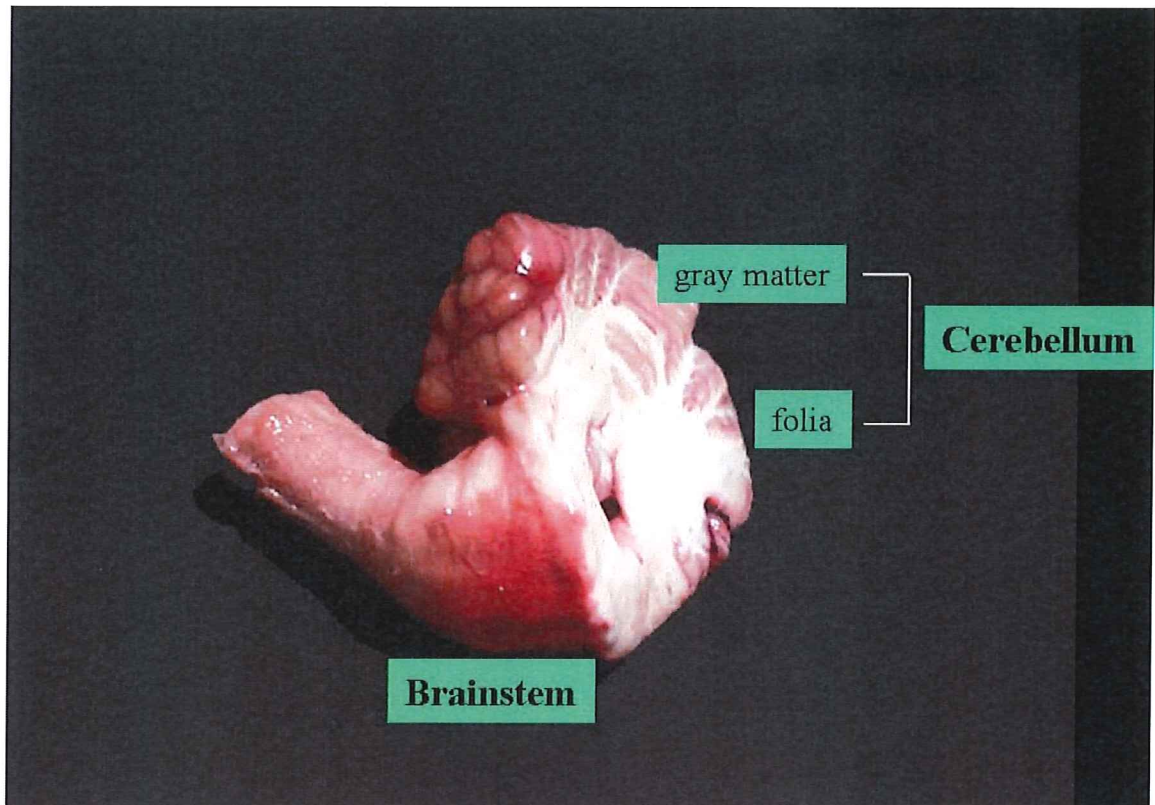
See figures 1 and 2 below for areas to test from the brain.

See links for rabies testing recommendations:

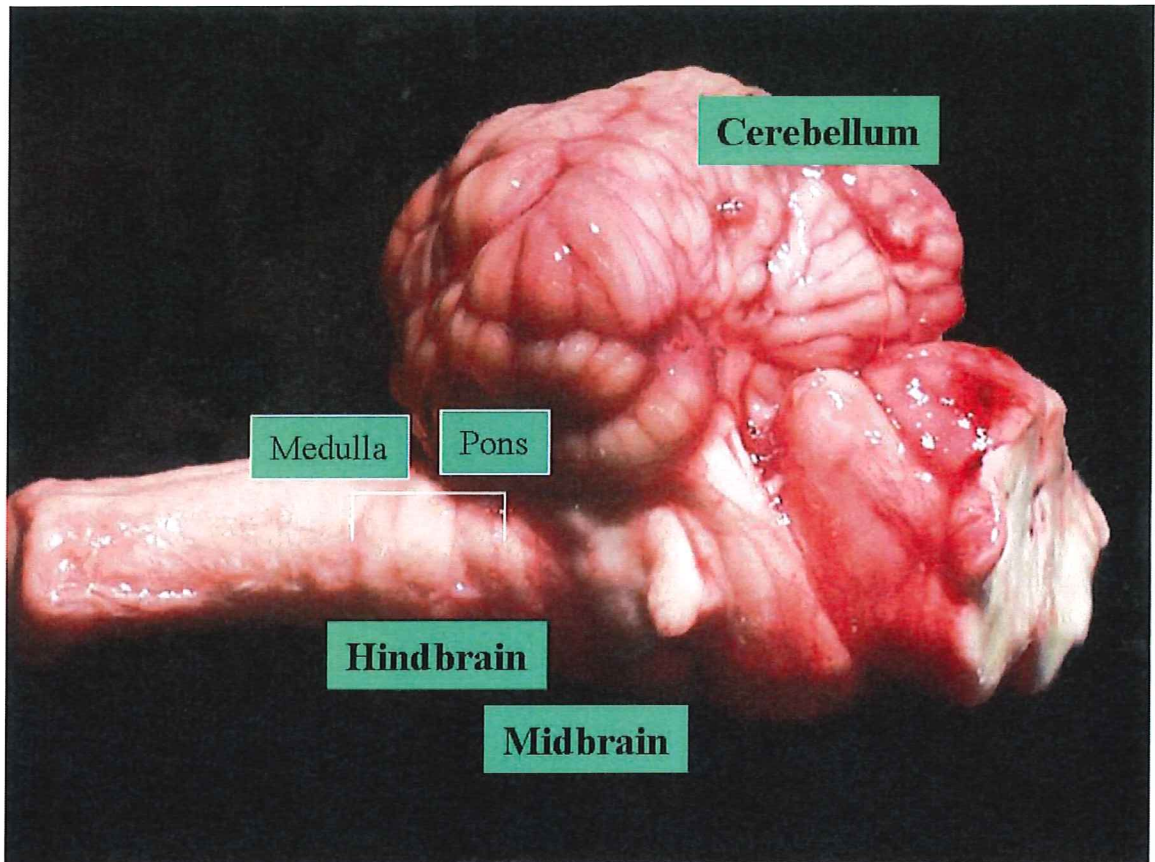
- [Rabies Laboratory Specimen Policy and Practice for New York State](#)
- [Centers for Disease Control and Prevention – Rabies Testing](#)
- Contact information for state and rabies consultation available [here](#).

Saliva and other body tissues become virus positive by the time of clinical signs, but there remains no fully reliable ante-mortem diagnostic test for rabies.

Personnel involved in the necropsy of a rabies suspect case should have had rabies prophylaxis and use appropriate barrier clothes for protection.



**Figure 1.** Surface of cut section through midbrain area showing convoluted gray matter and white foliar regions of the cerebellum, and the cerebellar connection to pons and medulla. Photo from link from CDC. [Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing](#)



**Figure 2.** Lateral view of brain with cerebrum removed to show the extension of brain stem beneath the cerebellum. A rabies diagnosis should include an observation of the cut surface of a cross section of the brain stem (through the medulla, pons, or midbrain area) and the cerebellum (through each hemisphere and the vermis). For example, a cross section of the midbrain area (dashed line) would include all tissues necessary for rabies diagnosis. Photo from link from the CDC. [Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing](#)

Send cross section of brain stem (through the medulla, pons, or midbrain area) and cerebellum (through EACH hemisphere and the vermis).

Many laboratories will not start ancillary testing until negative rabies results have been received. Contact the laboratory for specific policies and procedures.

#### **Post-mortem**

Rabies does not present with any gross lesions. Self inflicted wounds may be found on the body.

[Procedure to remove brain tissue \(Example from New York State\)](#)

NOTE: Practitioners performing necropsies in the field are encouraged to contact a veterinary diagnostic laboratory to which they plan to submit samples for further testing such as histopathology and pathogen identification in order to be certain they collect the appropriate samples and handle the samples in a manner that will optimize making a definitive diagnosis. For some situations such as neurologic cases submission of the entire carcass to the diagnostic laboratory for post-mortem examination is recommended due to the time and labor required to perform a complete exam and collection of samples from the equine CNS.

- Do NOT freeze rabies brain tissue samples; ship refrigerated in leak proof container so appropriate sections of brain can be identified by laboratory.
- It is appropriate to fix the remaining portion of the brain for eventual histological examination.
- Note: Some veterinary diagnostic laboratories will test for rabies on necropsy only when specifically requested to do so. Be sure to request rabies testing when submitting the carcass of any neurologic case for necropsy.

#### **Shedding Time of Organism Following Resolution of Clinical Signs**

N/A

#### **Environmental Persistence**

Rabies virus is sensitive to drying, ultraviolet radiation, and detergent. Rabies is inactivated and removed by standard decontamination practices which include washing instruments and environment with common disinfectants including quaternary ammonium compounds. Instruments should be sterilized by autoclaving.

#### **Specific Control Measures**

##### Rabies-suspect cases

- Minimize number of personnel in contact with suspect case.
- When possible, limit personnel to those having undergone pre-exposure immunoprophylaxis (vaccination).
- Gloves and protective eyewear must be worn by all in-contact personnel.
- Establish record of all individuals having handled horse beginning 48 hours prior to onset of clinical signs.
- Suspect horses should remain quarantined for 30 days.

### Prevention

Vaccination should be administered to horses annually after an initial series.

Previously vaccinated horses: Post-exposure prophylaxis should be performed promptly once exposure is suspected and the animal observed for 3 to 6 months.

Post-exposure vaccination of previously unvaccinated horses is of dubious value.

Feeding and/or housing of wild animals (as pets) are discouraged.

### **Release of Animals from Isolation**

Any horse exhibiting neurologic signs that has not been vaccinated against rabies should be considered a rabies suspect. Such horses should be handled using contact precautions, with records kept as described above. These precautions should remain in place for 30 days or until such time as an alternative diagnosis for the neurological signs are confirmed.

### **Biosecurity Issues for Receiving Animals**

N/A

### **Zoonotic Potential**

Yes. Identification of potential rabies-suspect cases is essential and should be promptly reported to public health authorities.

See [A Review of Equine Zoonotic Diseases: Risks in Veterinary Medicine](#)

*Note: This paper may only be viewed by AAEP members as it is only available within the members section of the AAEP website.*

# Salmonellosis

## Definition

Contagious bacterial infection caused by *Salmonella* spp. which has >2500 serotypes.

## Clinical signs

Diarrhea (soft feces to projectile, watery diarrhea)

Lethargy

Colic

Fever (patient may have normal temperature, especially if treated with NSAIDs)

Localized infection i.e. joint or bone

Endotoxemia

## Incubation

12+ hours (interval variation due to amount and virulence of organism ingested, immunocompetence, and other variables)

## Transmission

Ingestion of contaminated material or feces; inhalation *is* possible

Fomites are significant method of indirect transmission of these bacteria

## Diagnostic Testing

Fecal culture—request *Salmonella* specific with serotyping and antibiotic susceptibility testing of isolated organism.

An individual case should be sampled 3-5 times.

If several animals are affected, submit samples from as many animals as possible.

## Shedding Time of Organism Past Resolution of Clinical Signs

Highly variable, from several days to extended periods (30+ days)

Chronic shedding is uncommon but can occur.

## Environmental Persistence

Withstands freezing temperatures and can potentially survive *for years* under adverse environmental conditions.

Multiplies in temperatures ranging from 44.6-111° F (7-45° C).

Susceptible to chemical disinfection on *thoroughly cleaned surfaces*, but minute amounts can remain on porous surfaces and in hard-to-clean areas (drains, corners, beneath rubber matting, etc.) and remain a persistent source of infection.

## Specific Control Measures

### [Biosecurity Guidelines](#)

## **Release of Animals from Isolation**

For animals having positive cultures while clinically ill:

Fecal cultures for 5 consecutive days after resolution of clinical signs. When 5 consecutive samples test negative for *Salmonella* spp. the horse may be released from isolation.

**Note:** 5 consecutive negative samples do not guarantee the horse is 'free' of *Salmonella* spp. Rather they demonstrate that the horse was not shedding the bacteria at the time of the sample collections and are therefore suggestive it is no longer shedding.

## **Biosecurity Management for Receipt of Previously Infected Animals**

Isolate horse for 30 days from resident horses and re-test for 3-5 days of negative fecal cultures prior to releasing horse into general population.

Prior to entry into the general population the horse should be housed in an environment that can be cleaned and disinfected.

If the horse is turned out in a paddock, manure should be promptly removed and disposed of appropriately. Caretakers should wear protective boots. After the horse is released, the paddock should be harrowed and kept unused for 30 days.

## **Zoonotic Potential**

All *Salmonella* serotypes which cause disease in horses are potentially contagious to people.

Immuno-compromised individuals and children should be kept away from any animal with clinical disease suspected or confirmed to be *Salmonella* spp. and from any clinically normal animals suspected or confirmed to be shedding.

[Link to \*A Review of Equine Zoonotic Diseases: Risks in Veterinary Medicine \(J.S. Weese\)\*:  
http://www.aaep.org/proceedings/02proceedings/910102000362.pdf](http://www.aaep.org/proceedings/02proceedings/910102000362.pdf)

## Streptococcus equi var. equi

### Definition

*Streptococcus equi* is the etiologic agent for the upper respiratory disease commonly referred to as strangles. Less commonly, the bacteria may affect lymph nodes in the thorax and/or abdomen, causing a syndrome known as Metastatic strangles.

Comprehensive information is available in the ACVIM Consensus statement, '*Streptococcus equi* Infections in Horses: Guidelines for Treatment, Control and Prevention of Strangles', Corrine R. Sweeney, John F. Timoney, J. Richard Newton, and Melissa T. Hines, *J Vet Intern Med* 2005;19:123-134. Click here for link to ACVIM document: [www.acvim.org/uploadedfiles/Consensus\\_Statements/Strangles.pdf](http://www.acvim.org/uploadedfiles/Consensus_Statements/Strangles.pdf)

### Clinical Signs

Fever, usually preceding other clinical signs by 24-48 hours  
Lymphadenopathy +/- abscessation (retropharyngeal and submandibular LNs most commonly involved)  
Mucopurulent nasal discharge  
Pharyngitis  
Dysphagia  
Upper airway stridor

Clinical signs are age related, with older horses typically exhibiting milder signs of shorter duration.

### Incubation

3-14 days—shorter interval reflects exposure to larger bacterial challenge

### Transmission

Direct: horse-to-horse contact  
Indirect: fomites

### Diagnostic Testing

Bacterial culture—diagnostic test of choice for clinically affected horses  
Samples collected early in the course of clinical disease may yield negative results on culture. If signs are consistent with *Strep equi* infection, repeat testing at weekly intervals. If several animals are affected, submit single samples from as many animals as possible.

PCR—in combination with culture is test of choice determine the status of exposed and recovered animals  
More sensitive than culture to small amounts of bacterial DNA but does not differentiate live bacteria from dead  
False negative: PCR can be inhibited in presence of large amounts of mucopurulent debris

Sample collection:

Nasopharyngeal wash

Pass a sterile polypropylene catheter (8-10 fr.) through the ventral nasal meatus until resistance is met (approx 10-15 cm). Flush 60 ml of warmed sterile saline through the catheter. Catch reflux fluid that drains from both nostrils into sterile container. Refrigerate sample; do not freeze.

Wear disposable exam gloves; change after each horse

Disinfect twitch, lead shank, lip chain after each horse

If washes are being performed on multiple horses, exercise caution to avoid cross-contaminating the exteriors of collection containers.

\*\*Endoscopic exam and testing of both guttural pouches should be performed on any horse for which a positive PCR and/or culture is reported by the laboratory. [Click here for photos of endoscopic findings of guttural pouches.](#)

#### SeM-specific ELISA

Cannot differentiate antibodies due to natural infection from those induced by vaccination, therefore of limited use in managing disease outbreaks, but may be useful for identification of animals requiring booster vaccination.

### **Shedding Time of Organism Past Resolution of Clinical Signs**

Typically, 2-3 weeks post-recovery but intermittent shedding may occur for months to years when bacteria persists in guttural pouches or paranasal sinuses.

Endoscopic examination and sampling (for culture and PCR) of the guttural pouches is warranted in detection of persistent infection.

Absent of diagnostic testing to detect chronic shedders, horses should be considered infective for up to 6 weeks post -infection.

### **Environmental Persistence**

Reports of environmental viability vary widely. Aggressive cleaning and disinfection, with special attention to the cleaning and disinfection of water containers, feeders, fences, stall walls and trailers, is indicated. It is recommended that pastures and paddocks be rested at least 30 days.

### **Specific Control and Treatment Measures**

#### **Biosecurity Guidelines**

##### Disease surveillance

Recording rectal temperatures twice daily with segregation and initiation of testing on any horse developing fever > 102.5° F (39° C) or clinical signs.

Clinically normal horses housed within the primary perimeter may be permitted segregated exercise periods outside the perimeter. Precautions should be taken, and may include:

Exercise scheduled *after* general population's exercise period to avoid potential bacterial transfer to unaffected horses/barns by exercise riders

Access to starting gate or similar equipment denied

Restricted use of ponies/out-riders' horses—horses housed within the primary perimeter may only be escorted by horses housed within the same facility.

Direct horse-to-horse contact is to be avoided

Prompt post-contact use of hand sanitizer by individuals having contact with horses during exercise

### **Release of Animals**

To minimize the risk that recovered horses may pose, 3 consecutive weekly PCR and culture by nasopharyngeal wash are recommended. Should one of these tests result in a positive, it is advisable that further diagnostic investigation be performed to locate the focus of persistent infection. Treatment, with subsequent retesting, is appropriate.

Note: Thorough cleaning and disinfection of endoscope is critical to generation of reliable test results and prevention of disease spread.

For animals having been housed within the secondary perimeter:

Release testing is unnecessary in clinically normal horses having no history of exposure, and having had normal rectal temperature for 21 days.

### **Biosecurity Management for Receipt of Animals**

Requirements for accepting animals are determined after identifying ‘acceptable level of risk’ for the recipient facility. [View Pre-Outbreak Considerations.](#)

Given the mobility of populations involved in showing/racing/competition, exposure risk cannot be completely eliminated. The following options may be considered:

For horses having been housed within primary perimeter:

3 consecutive weekly nasopharyngeal lavagesamples tested by PCR and culture with negative results

For other horses:

Certificate of Veterinary Inspection with disease-specific endorsement:

*Horse(s) represented on the certificate of Veterinary Inspection have not originated from premises under quarantine for Strep equi, nor have been exposed to a confirmed or suspect case of Strep equi, nor have shown clinical signs suggestive of Strep equi infection, nor have been febrile within the previous 21 days.*

Vaccination—While it may be advisable to *recommend* vaccination, specific vaccination decisions should remain the purview of the attending veterinarian.

Single negative PCR/culture—of little value as a stand-alone indicator of risk, and must be evaluated in the context of exposure history.

### **Zoonotic Potential**

Human cases have been reported, but are uncommon. Immuno-compromised individuals should take precautions to avoid exposure.

# Arboviruses

## Definition

Arboviruses (arthropod borne viruses) of significance to the equine population are:

**Eastern Equine Encephalitis (EEE)**

**Western Equine Encephalitis (WEE)**

**Venezuelan Equine Encephalitis (VEE)**

**West Nile Virus (WNV)**

EEE, WEE, and VEE are alphaviruses. West Nile virus is an arbovirus but is unrelated to alphaviruses.

## Alphaviruses

**Eastern Equine Encephalitis virus (EEE):** The focus of this disease is usually the East coast with cases occurring as far west as Ohio. The mosquito vector for EEE includes members of the *Aedes* sp. and *Coquillettidia perturbans*.

**Western Equine Encephalitis virus,** as the name suggests, principally occurs in the Midwest and Western United States. While large equine outbreaks have occurred, no equine cases were reported from 1999-2006. The most important vector of this disease is *Culex tarsalis*.

**Venezuelan Equine Encephalitis virus** has a geographical distribution restricted predominantly to Central and South America, although U.S. incursions have occurred, and the risk of introduction persists. Several efficient vectors have been identified and these include common genera, *Aedes*, *Anopheles* and *Culex* spp. *VEE is a reportable disease; consult State Veterinarian when disease is suspected.*

## Clinical Signs—highly variable; none pathognomonic

Moderate to high fever 102.5-104.5°F (39.17-40.28°C)

Depression/somnolence

Inappetance

Diarrhea (VEE only)

Dysphagia

Head pressing

Circling

Blindness

Dementia

Seizures

Rapid personality change: somnolence, hyperexcitability, mania, self-mutilation

Cranial neuropathy: nystagmus, facial nerve paralysis, and weakness of the tongue and pharynx

Coma

Death

Mortality:

EEE 75-95% (usually within 2-3 days of onset of signs)

WEE 20-40% (WEE affects horses less severely than EEE)

VEE 40-90%

Survivors: Alphavirus infection can result in long-term abnormalities in horses that survive. Horses can exhibit abnormal mentation and/or residual spinal cord abnormalities. Horses affected by EEE and VEE are most likely to exhibit these signs.

## Incubation

EEE: 3 – 7 days

VEE: 2 – 4 days

## Transmission

Indirect, bites from mosquitoes having become infected with virus while feeding on viremic animal or avian host. Multiple animal species may seroconvert or develop clinical signs, however only those that develop significant viremia are reservoir hosts.

**EEE/WEE/WNV:** Only birds appear to develop a significant level of virus in their blood and can thus transmit the respective disease. An infected horse is a dead-end host; viral exposure can occur through necropsy procedures and handling of tissues. Appropriate precautions should be taken.

**VEE: Horses do develop a high enough viral titer to transmit to mosquitoes, therefore, horses have a reservoir role in VEE infections. During epizootics, horses are important amplifiers of the virus.**

## Diagnostic Testing

MAC- ELISA test—single serum sample (red top tube)

Results of >1:400 are confirmatory in horse(s) exhibiting clinical signs consistent with EEE/WEE.

Paired serum neutralizing antibody titers: 2 samples (red top tube) collected 2-4 weeks apart

Four-fold increase in titers between samples is considered confirmatory in horse(s) exhibiting clinical signs consistent with EEE and not having been recently vaccinated.

CSF analysis:

CSF WBC count—usually > 7 cells/ul

CSF total protein—usually >70 mg/dl

PCR may be attempted on CSF of clinically affected horses. Test sensitivity is limited.

Note: CSF fluid requires prompt evaluation. When this is not feasible, direct smear slide mounts may be made and express shipped on cold packs to the laboratory.

Post-mortem:

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.

**Post-mortem sample collection requires appropriate precautions to avoid exposure.**

[Click here for necropsy procedures.](#)

Histopathology: Fix at least one-half of the brain for histopathology. Fresh brain should be submitted for concomitant virus isolation, immunochemistry, and rabies testing. [Click here for brain removal document.](#)

## Shedding Time of Organism Past Resolution of Clinical Signs

VEE horses develop significant viremia between 2 and 5 days after infection. Horses do not shed this virus, but mosquitoes can obtain virus by ingesting a blood meal from these horses.

## **Environmental Persistence**

This enveloped virus is susceptible to drying, ultraviolet light, and detergent.

## **Specific Control and Treatment Measures**

### Vaccination

After initial vaccination series (per manufacturer's instructions), administer annual booster prior to mosquito season. In endemic areas, booster at 4 month intervals during mosquito season.

### Vector control

## **Release of Animals from Isolation**

No restrictions need be placed on recovered animals.

## **Biosecurity Issues for Receiving Animals**

None.

## **Zoonotic Potential**

Human risk of exposure via bite of infected mosquito or through handling CNS tissue and/or fluids of infected animal. Precautions are indicated when performing necropsy examinations of neurologic horses.

Link to *A Review of Equine Zoonotic Diseases: Risks in Veterinary Medicine (J.S. Weese)*:  
<http://www.aaep.org/proceedings/02proceedings/910102000362.pdf>

# West Nile Virus

## Definition

West Nile virus (WNV) is a mosquito-borne flavivirus that was first diagnosed in North America in the New York City area in 1999.

WNV primarily causes disease in birds, humans and horses and is transmitted by many different species of mosquitoes.

Since 1999, over 16,000 U.S. horses have been confirmed for WNV encephalomyelitis.

During 2002, over 15,000 horses were affected with an approximately 30% mortality rate.

WNV is now considered endemic with yearly activity in the United States, Canada, Mexico and the Caribbean.

## Clinical Signs

Mild low-grade fever 101.9-103.5°F (38.83-39.72)

Inappetance

Lethargy/somnolence

Neurologic signs

Onset of neurologic disease is frequently sudden and progressive and characterized by problems in maintaining balance and strength.

Periods of hyperexcitability, apprehension and/or somnolence

Fine tremors and fasciculations of the face and neck muscles

Cranial nerve paralysis-- facial paralysis and weakness of the tongue very common

Complete paralysis of one or more limbs

Expect clinical signs to be most severe in the young and old

About 1/3 of WNV cases experience an increase in severity of clinical signs within 7-10 days of onset, sometimes after initial clinical signs have abated.

## Incubation

7-10 days

## Transmission

Mosquito vector

Only birds appear to develop significant levels of virus and serve as a source of infection.

An infected WNV horse is not infectious and poses no risk to other horses, humans or birds.

## Diagnostic Testing

MAC-ELISA test: Single serum sample (red top tube)

Positive test: Antibodies detected at or above 1:400 serum dilution

A positive test is considered confirmatory in horse exhibiting clinical signs consistent with WNV.

Plaque Reduction Neutralization Test (PRNT) paired serum antibody titers

Collect samples 2-4 weeks apart (red top tube)

Four-fold change in PRNT titers between samples is considered confirmatory in horse exhibiting clinical signs consistent with WNV in horse that has not been vaccinated recently.

Send serum overnight at refrigerator temperature on a cold pack to laboratory.

CSF Analysis: elevated mononuclear cell count and/or high total protein

CSF WBC Count: Highly variable range, when abnormal, CSF WBC is usually  $\geq 7$  cells/ul.

Protein Level: Highly variable range, when abnormal, CSF protein is usually  $\geq 70$  mg/dl.

PCR can be attempted on CSF of clinically affected horses, however, there is limited sensitivity.

Note: CSF fluid should be evaluated immediately. If evaluation cannot be performed, a direct smear can be made and the CSF and slide sent to laboratory overnight at refrigerator temperature on a cold pack.

#### Post-mortem

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.

**Post-mortem sample collection requires appropriate precautions to avoid exposure.**

[Click here for necropsy procedure for suspected cases of zoonotic disease.](#)

Histopathology: Fix at least one-half of the brain for histopathology. Fresh brain should be submitted for concomitant virus isolation, immunochemistry, and rabies testing. [Click here for document on brain removal.](#)

### **Shedding Time of Organism Past Resolution of Clinical Signs**

While horses do become mildly viremic 3-5 days post challenge, they do not shed virus nor do they develop adequate viremia to serve as a source of virus to insect vectors.

### **Environmental Persistence**

This enveloped virus is susceptible to drying, ultraviolet light, and detergent.

### **Specific Control Measures**

#### **Prevention**

Vaccination—killed or modified live vaccine:

Initial injection of either vaccine is followed in 3 to 6 weeks with a booster. The primary series must occur to elicit optimal antibody production. Vaccination should be initiated before the mosquito season as data illustrates that the development of neutralizing antibody response is slow. It is not expected that the initial series will provide long-term protection.

Vaccine manufacturers recommend subsequent boosters be administered twice yearly in epidemic or endemic areas.

Many pregnant mares have been safely vaccinated with currently marketed products. {Vest, D., Cohen, N., Berezowski, C., et al., JAVMA 225(12) December 15, 2004, 1894-1897.}

No information is available regarding long-term immunity.

#### Vector management

Use insect repellents frequently; re-apply after rain.

Keep horses in at night when possible, or apply insect repellent.

Use fans and air movement to decrease mosquito feeding activity.

Eliminate or minimize standing water.

Stock tanks or ponds should be stocked with mosquito-feeding fish.  
Eliminate brush piles, gutters, old tires and litter.  
Remove all equipment in which standing water can collect.

### **Release of Animals from Isolation**

No restrictions need be placed on affected or recovered animals.

### **Biosecurity Issues for Receiving Animals**

None.

### **Zoonotic Potential**

While not a source of virus to insect vectors, an equine case of WNV may be an early warning signal to humans that there are infected mosquitoes present.

The most serious risk to humans in regards to WNV horses is during work with WNV-infected brain and/or cerebrospinal fluid. Appropriate barrier clothing must be worn and exposure precautions taken.

*Link to A Review of Equine Zoonotic Diseases: Risks in Veterinary Medicine (J.S. Weese):*  
<http://www.aaep.org/proceedings/02proceedings/910102000362.pdf>