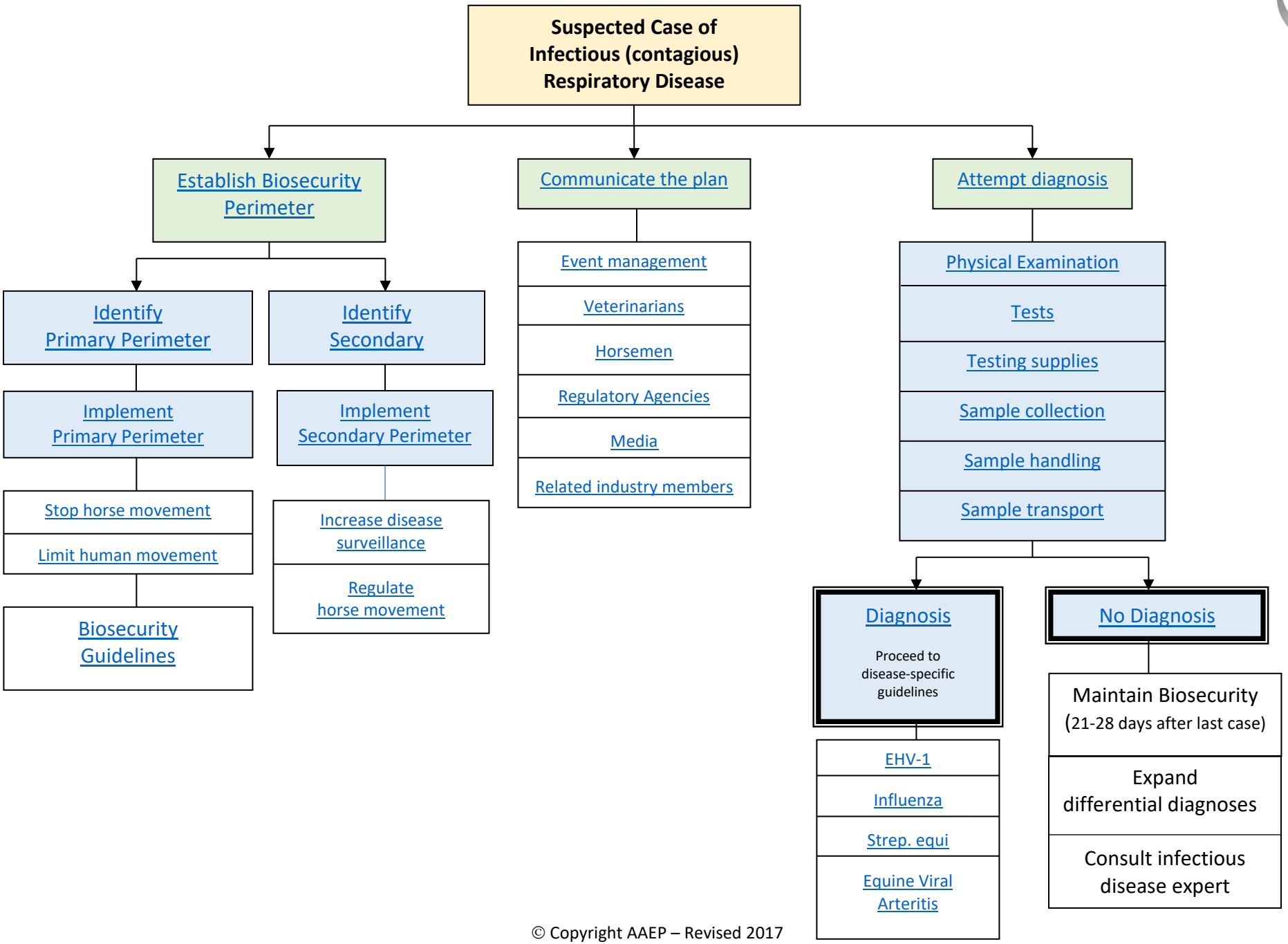
AAEP Guidelines: Suspected Case of Infectious Respiratory Disease



Differential Diagnoses

Primary agents (US based horses) having herd health implications:

- Influenza
- Equine Herpesvirus-1 (EHV-1)
  - ACVIM Consensus Statement
- Equine Herpvesvirus-4 (EHV-4)
- Equine Arteritis Virus (EAV)
- Equine Rhinitis A and B infections
- Equine Adenovirus Infections
- *Streptococcus equi* subspecies equi
  - ACVIM Consensus Statement

Additional differential diagnoses for respiratory disease should be considered for imported horses, or show and racehorses that travel across borders or internationally.

Establish Biosecurity Perimeter

Upon suspicion of a contagious respiratory disease, biosecurity procedures and protocols should be immediately implemented.

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

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. Thermometers should be specific to each horse and not transferred
  - 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.

Implement Secondary Perimeter

- ***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
- Personnel Management
  - Requirements
  - Instructions
  - Notification of zoonotic risk, if pertinent
- Outbreak updates
- Event Management Biosecurity Resources:
  - Biosecurity Tool Kit for Equine Events

II. Veterinarians

- Biosecurity Guidelines
- 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

Diagnosis should be attempted during the initial physical examination. This will decrease the risk of disease spread that occurs with multiple interactions with the infected horse and its environment. Prior to obtaining diagnostic samples, the diagnostic lab should be contacted to ensure the proper samples are obtained and they are handled appropriately. Specifics on diagnostics are outlined at the end of this section.

Diagnostic Sampling, Testing, and Handling

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 ELISA (enzyme linked immunosorbent assay) or fluorescent antibody tests (FAT).

- 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**

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.

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 a 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, EVA, and Strangles. Emerging pathogens to consider including are EHV-2, EHV-5, equine rhinitis virus A and B, and equine adenovirus 1 and 2. There are respiratory panels that include most if not all the possible pathogens.

- Virus isolation: Influenza, EHV 1&4, EVA, Equine Rhinitis A & B infections, Equine Adenovirus infection
- PCR: Influenza, EHV 1&4, EVA, Strangles
- Bacterial culture: *Strep equi*
- Some laboratories offer 'respiratory panels' at reduced financial cost

Sampling

Where viral pathogens are suspected:

- All swabs and small tissue specimens for viral isolation should be placed in 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. If viral transport media is not available, swabs may be placed in a plain red top tube with 1–2 drops of sterile saline to maintain moisture for possible viral isolation**
- 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 be at least 6 inches long in order to reach far enough into the nose for a satisfactory sample. The long swabs that can reach the nasopharynx may be resented by some horses
- Use of inappropriate sample collection materials will compromise reliability of test results

Where bacterial pathogens are suspected:

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); type "viral transport" into the search box for several choices.

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

Sample Collection

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. Subclinically infected chronic carriers can shed intermittently

Sample collection in all cases of suspected infectious respiratory disease:

- Wear personal protective equipment: disposable exam gloves, plastic gowns where clothing may be contaminated, plastic boots. Change between horses. Collect and dispose of used equipment in a manner to avoid spread of infectious agents
- 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. If no viral isolation media is available, samples can be placed in plain red top tube with 1–2 drops of sterile saline to keep sample moist.

- Collect the swab from the ventral meatus, ensuring enough restraint for the swab to be held against the mucosa for at least 10 seconds. (See link for obtaining nasal swabs)
- Immediately place in a cooled container prior to transport to the laboratory. (Some labs prefer more than one swab per horse

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 a 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 delivered to the diagnostic laboratory as fast as possible to ensure arrival in suitable 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. Nasal swab samples for viral testing may be frozen, but samples submitted for bacterial culture (i.e. *Strep equi*) should be refrigerated and shipped refrigerated by overnight courier. If dry ice is used for freezing specimens, the samples must be kept in airtight plastic bags or sealed containers (CO_2 released from dry ice is harmful to viruses)

Post-mortem tissue samples

Collect small blocks of lung tissue (1–2 cm^3) 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.

Fixed tissues should also be submitted for histological examination. Tissue samples of 0.5 cm^3 should be placed in 10% formalin. Diagnostic testing may be progressively undertaken and histological samples may be tested if other tests are negative or inconclusive as the discretion of the veterinarian.

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 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:

- Influenza
- EHV-1
- Strep. equi

No Diagnosis

- EVA

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