



EQUINE INFLUENZA (EIV)

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Definition

Equine influenza virus (EIV) is an RNA virus endemic to horse populations in many countries worldwide. Equine influenza outbreaks may also occur sporadically in epidemic form. Countries historically free of equine influenza include Iceland and New Zealand. While epidemic outbreaks have occurred in Australia, Japan, and South Africa, these countries are currently considered equine influenza-free.

The role of the Office International des Epizooties (OIE)

The OIE, or World Organization for Animal Health, is an intergovernmental organization with 182 member countries (including the USA), with the goal of improving animal health worldwide. It sets guidelines for combatting selected animal diseases of international significance (OIE-listed diseases). Equine influenza is an OIE-listed disease. Under the auspices of the OIE, a scientific committee of equine influenza experts meets annually to review equine influenza activity around the world, to characterize the viruses responsible, and to make recommendations for equine influenza vaccine strain composition. Their conclusions and recommendations are published annually in the OIE Bulletin. The OIE also publishes the Terrestrial Code, which makes recommendations concerning equine influenza vaccination, quarantine, and testing protocols for horses being shipped internationally, with particular attention to equine influenza-free countries.

Equine influenza virus has historically existed in 2 forms: the H7N7 or equine-1 subtype, and the H3N8 or equine-2 subtype. The equine-1 subtype is believed to be extinct and is no longer recommended for vaccines. The equine-2 subtype undergoes a gradual process of change called antigenic drift, due to accumulating mutations in its major surface antigens, the hemagglutinin (H or HA) and neuraminidase (N or NA). This drift sometimes leads to the co-existence of virus strains belonging to lineages that once were very similar but have drifted to become increasingly dissimilar, different enough to make cross-immunity ineffective. That is, a given horse's level of immunity to equine influenza might be sufficient to protect against one lineage but not the other. Because horses travel internationally, this necessitates the inclusion of representative strains from both viral lineages in equine influenza vaccines. Currently there are 2 such co-circulating lineages, called Florida clade 1 and Florida clade 2, that split apart around the year 2000 and have been drifting apart ever since. A vaccine against one lineage does not guarantee protection against the other, and both are recommended for inclusion in vaccines. For many years the Florida clade 1 lineage dominated the equine



influenza virus circulation in the USA and the Florida clade 2 lineage dominated in Europe. In 2018-19, Florida clade 1 caused large outbreaks in Europe as well.

Clinical Signs

Clinical signs vary in their severity depending on the age and immune status of the horse, and asymptomatic infection is possible. Clinical signs are more common and often more severe in younger horses; ages 1-5yo. Older horses generally have milder disease.

- Fever, up to 106F (41.1C), depression, malaise, anorexia, muscle pain/weakness
 - Dry, harsh cough (sometimes paroxysmal) usually precedes fever. The dry, harsh cough is a frequent clinical sign in EI while it is less common with EHV infection. Cough can last up to 6 weeks after all other clinical signs have abated. Can take up to 6 months to regain previous athletic abilities
 - Mild enlargement of retropharyngeal lymph nodes
 - Serous nasal discharge that frequently progresses to mucopurulent with onset of secondary bacterial infections
 - Secondary bacterial infections are very common in influenza-affected horses
 - Rarely, clinical signs may include distal limb edema and cardiomyopathy.
- May be more severe in donkeys and mules

Incubation Period

The period between exposure to EIV and appearance of clinical signs is frequently as short as 24 hours and may be up to 3 days.

Risk Factors

- Age: horses 1-5 years of age
- Areas of high comingling of horses such as racetracks, show grounds, veterinary hospitals
- Immunosuppression from traveling, hospitalization, training and showing
- While vaccination reduces the risk of clinical disease, vaccinated horses can still become infected and shed virus (subclinical shedding)

Transmission

- Respiratory transmission occurs most commonly through inhalation of infective droplets from coughing and snorting horses. The distance these droplets may spread through the air has not been definitively established, but may be as far as 50 yards.
- Indirect transmission can occur and can be an important means of spread. This includes transmission of the virus on contaminated clothing, equipment, brushes, shared water buckets, hands, etc.
- Respiratory shedding typically lasts for 7-10 days post infection in naïve animals; much shorter shedding periods occur in partially immune (previously vaccinated) horses.

Diagnostic Sampling, Testing and Handling

Virus isolation from nasopharyngeal swabs

- Samples should be collected within 24-48 hours after onset of clinical signs.
- Swabs should be submitted in viral isolation transport media (NOT bacterial transport media). If no viral transport media is available, place swabs in a red top tube with a few sterile saline drops (enough to moisten the tip of the swab). Ship overnight on ice.



- **Nasopharyngeal swabs are a superior alternative to nasal swabs**, in that these collect about 10 times as much virus as nasal swabs which reduces the possibility of a false-negative diagnostic test result. These are also safer in that there is no chance that a horse movement plus inhalation might cause the swab to be lodged inaccessibly within the nasal meatus.
- Nasopharyngeal swabs are typically 40cm long, with Rayon gauze tips similar to nasal swabs but larger. These are commercially available from Medline Inc. or Birchwood Laboratories Inc.
- **Nasopharyngeal swabbing technique:** Holding the swab at least 10 cm from the gauze tip to avoid contamination, insert the swab into the nostril, angling inwards and down so as to avoid the dorso-medial blind pouch inside the nostril, then pass the swab as far as possible down the ventral nasal meatus to the nasopharynx, (about 25 cm for an adult horse, 20 cm for a pony, and 15-20 cm for a foal). Rotate 3-4X, then remove. The gauze tip will need to be cut off with scissors to fit within the transport tube. That transport tube should contain enough viral transport media (or sterile normal saline) to completely immerse the gauze tip.

Real time PCR (RT-PCR) from nasopharyngeal swabs

- Only detects certain strains, i.e., H3N8 or H1N1.
- Swabs should be submitted in viral isolation transport media (NOT bacterial transport media). If no viral transport media is available, place swabs in a red top tube with a few sterile saline drops (enough to moisten the tip of the swab). Ship overnight on ice.
- EDTA blood is NOT an acceptable sample
- Additional information available at Cornell Animal Health Diagnostic Center: <https://www.vet.cornell.edu/animal-health-diagnostic-center>

Serology

- Paired (acute and convalescent) sera can be very useful in confirming a diagnosis of equine influenza. Acute sample should be obtained as close to onset of clinical signs (max of 3 days) as possible and convalescent sample should be collected 2 weeks later.
- Serology can confirm infection even in the face of a false negative virus isolation.
- Submit separated serum samples (clot must be removed) in a red top tube. Serum samples are stable at room temp for several days; longer requires refrigeration or freezing

Immunoassay (stall-side kit)

- There are several available products with varying diagnostic reliability

Postmortem

It is very rare that equine influenza infection would result in a fatal outcome. Thus, there are few reports of gross pathologic findings. Based on original studies of influenza, changes include bronchiolitis, peribronchiolitis and subacute interstitial pneumonia.



Shedding of Organism Following Resolution of Clinical Signs	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.
Environmental Persistence	<ul style="list-style-type: none"> • Respiratory shedding typically lasts for 7-10 days post infection in naïve animals; much shorter shedding periods occur in partially immune (previously vaccinated) horses. The period of live virus shedding does not always correspond to clinical illness, and horses may continue to shed virus after resolution of fever and clinical signs. Virus may be detectable by PCR at 15 days or longer post-infection. • Virus can remain viable for up to 2 days on contaminated fomites and solid environmental surfaces, e.g. grooming supplies, stall latches, etc. • Virus can survive in aerosols for several hours and on hands for a few minutes • In water, virus viability has been reported up to 3 days. Virus survival in water is temperature dependent and may be longer than 3 days in cold water.
Specific Control Measures	<p>Vaccination</p> <ul style="list-style-type: none"> • AAEP Equine Influenza (EIV) Vaccination Guidelines • While annual vaccination is currently recommended, more frequent vaccination may be recommended for young horses and horses at increased risk due to environmental and/or management factors • In an outbreak situation, booster vaccination of unexposed healthy animals is likely to be of value if administered at least 10 days prior to exposure. Such vaccination is not known to result in complications. • If animals are unvaccinated prior to an outbreak, the use of a modified live intranasal vaccine may be recommended to achieve protection within 5 days of primary administration. <p>Isolation and Biosecurity</p> <ul style="list-style-type: none"> • AAEP Biosecurity Guidelines • Any horse showing clinical signs of any respiratory disease (coughing, nasal discharge, fever) should be immediately isolated and standard respiratory biosecurity guidelines should be followed until a diagnosis is confirmed. • Horses housed in the same barn with a horse showing respiratory clinical signs should be isolated for 14 days. Because many horses in an individual barn may be affected simultaneously or are incubating infection when the first case is recognized, it may be best to isolate all horses together by quarantining the entire facility rather than moving them to individual isolation facilities. All horse movement on and off the premises should be suspended during the quarantine period. • During an influenza outbreak, affected and exposed animals should be isolated from susceptible horses, preferably in a different air space. Coughing horses can aerosolize the virus and transmit infective virus particles for 35 yards, and potentially farther depending on housing conditions and ventilation.



<p>Biosecurity Issues for Receiving Animals</p>	<p>Release of animals from isolation</p> <ul style="list-style-type: none"> • Maintain quarantine and isolation procedures (primary perimeter) for 14 days <u>after</u> resolution of last suspected case • If horses are in pre-export isolation, the OIE Terrestrial Code recommends that this period should be 21 days and that horses must remain free of disease throughout. <ul style="list-style-type: none"> • Isolate all horses returning from shows, exhibitions, or trail rides for 10-14 days. • Consider vaccination requirement for facilities with elevated risk
<p>Disinfection</p>	<p>EIV is easily killed by many commonly used disinfectants. Antec Virkon™ S with potassium peroxymonosulfate and sodium chloride kills EIV regardless of time, temperature or presence of organic matter. Alcohol-based hand sanitizers are effective against influenza viruses.</p>
<p>Zoonotic Potential</p>	<p>None known, but strains of equine H3N8 influenza virus can infect canines.</p> <p>There is evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. While equine influenza has not been shown to cause disease in humans, serological evidence of infection has been described primarily in individuals with an occupational exposure to the virus. There is little risk to public health. In experimental settings, the virus has shown the ability to infect humans, and a few people in contact with infected horses developed antibodies to equine influenza viruses, but no humans exposed to the virus have become ill.</p>
<p>Further Reading</p>	<p>Equine Influenza Info - World Organisation for Animal Health (OIE) https://www.oie.int/en/animal-health-in-the-world/animal-diseases/Equine-influenza/</p> <p>Paillot, R. et al. <i>Duration of equine influenza virus shedding and infectivity in immunised horses after experimental infection with EIV A/eq2/Richmond/1/07</i>. Vet Microbiol 166, 22-34 (2013).</p> <p>Chambers, T.M. and Reedy, S.E. Ch.32: <i>Equine Influenza Diagnosis: Sample Collection and Transport</i>. In. E. Spackman (ed.) Animal Influenza Viruses. Methods in Molecular Biology v.1161, pp. 371-377, Springer, New York, 2014.</p>

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