Update on Infectious Equine Respiratory Disease

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Brief immunology overview of the equine respiratory system
-Respiratory Immunoglobulins, Alveolar macrophages, Lymphoid Tissues
-General immunological status of the lung
-Introduction of infectious agents and the acute inflammatory response

The overall importance of immunology in respiratory disease cannot be underestimated. It allows for an understanding of both the development of disease and provides a framework by which it may be prevented. Additionally, the clinical manifestations of respiratory disease are not due solely to the invading pathogen or antigenic stimulus but also from0 the host's immunological response to the offending agent. Clearly, prevention is far more effective than any treatment that may be administered following the development of disease.

The equine respiratory system handles about 100,000 liters of air during a 24 hour period. Initial filtering of this air is performed by the nasal passages but many particles (up to 10 microns) will pass through these defenses and impact the upper airway (trachea). Smaller particles (1-5 microns) including bacteria and viruses, will reach the lower airway and contact a host of cellular defense mechanisms. Environmental factors, including transport, housing, hay quality etc. may greatly increase respirable debris and exposure to infectious agents. Thus, the respiratory tract is constantly exposed to potential antigenic material and infectious agents. Under 'normal' conditions the defense mechanisms remove these particles prior to development of infection or an inflammatory response.

For respiratory disease to occur, 3 criteria necessary:
1. Host
2. An agent (infectious, toxic...)
3. Appropriate environment (including both where the animal lives and local conditions within respiratory tract)

Disease is often the result of interaction of one or more agents (virus, bacteria) in a stressed horse (compromised immune response) in an adverse environment.

Incidence and pathogens of Equine Respiratory Disease
Viral diseases involving the respiratory tract of horses have been identified as one of the most common problems encountered by equine veterinarians. Equine herpesvirus types 1 and 4 (recently EHV-2 and 5), and equine influenza are among the most frequently recognized viral pathogens. Several studies (dependent upon country, age of horses, surveillance methods and diagnostic molecular tools) cite an incidence of 1.5 to 26.4% with EHV-4 as the most common viral pathogen detected in horses with upper respiratory tract disease. Infections with influenza virus and EHV-4 have demonstrated the ability to significantly decrease mucociliary clearance for up to 30 days. Horses typically acquire bacterial pneumonia by aspiration of microorganisms that normally inhabit the upper airway. By far, the most common pathogen isolated from horses with pneumonia is *Streptococcus equi* subspecies *zooepidemicus.*

Regardless of the mechanism predisposing horses to bacterial colonization (virus + stress/compromised immune response) the inflammatory response triggered by microbial invasion results in neutrophilic infiltration and the subsequent release of inflammatory mediators that ultimately damage the airway/capillary epithelium resulting in pneumonia.

Equine Influenza Virus

Clinical relevance

EIV is a common equine respiratory virus, and is responsible for outbreaks in all horse populations. EIV is an orthomyxovirus, and is categorized according to its hemagglutinin (HA) and neuraminidase (NA) proteins. The most common EIV substype is the H3N8 strain. EIV, as with human and other influenza viruses, changes these epitopes according to host immune pressure. The resulting "antigenic drift" can make it difficult for vaccine manufacturers and the horse's natural immunity to keep pace with the virus.

Epidemiology

Although all equine age groups are susceptible to EIV, most foals are protected by maternal antibodies until 5-6 months of age. Older horses have at least partial immunity due to natural exposure or vaccination. Fortunately for the virus, and unfortunately for the host, EIV has an extremely short incubation period and is shed within two days of exposure. This may well precede any clinical recognition of disease. Because EIV can cause protracted coughing in the horse, it is readily spread in aerosols.

Pathogenesis

EIV is frequently designated as a pathogen of the upper respiratory tract, but the most significant pathologic lesions appear in the lower respiratory tract. The virus has a tropism for respiratory epithelium, which it destroys, leading to severe impairment of mucociliary clearance. Necrosis of the epithelium also paves the way for secondary bacterial invaders such as *S. equi* subsp *zooepidemicus*, leading to pneumonia. In managing the convalescent horse, it is important to remember that it takes a minimum of 3 weeks to repair the respiratory epithelium.

Diagnosis

Although the clinician may have strong suspicion of EIV based on history and clinical signs, more rigorous diagnostic methods are necessary to distinguish this disease from other equine viral respiratory infections. The Directigen™ Flu-A test (Becton Dickinson) is a stall-side assay to detect the presence of influenza A virus, but does not characterize the virus further. Similarly, paired serology will confirm an influenza virus infection, but virus isolation is important in helping to determine the epidemic variant, and thus help to formulate future vaccines. Virus isolation is markedly improved if proper viral transport medium is used for sending the sample to the lab. Bacterial overgrowth can otherwise destroy the sample. PCR detection is also available from diagnostic laboratories as a rapid-detection method for identifying EIV H3N8 as well as other EIV subtypes. Clinical samples for PCR assays are obtained from nasopharyngeal swabs and submitted in transport medium, similar to what is done with virus isolation samples. PCR has the advantage of increased sensitivity compared to virus isolation methods.

<u>Diagnostic Testing for Influenza: summary</u>

Virus Isolation, Rapid Cell Culture, Immunofluorescence, Rapid Influenza Diagnostic Test (RIDT), ELISA , RT-PCR, SRH, Hemagglutinin Inhibition (HI)

<u>Rapid Influenza Diagnostic Test</u>

1. Stall side testing
2. Tests for Type A and Type B Influenza
3. Very Sensitive – picks up influenza DNA
4. Uses nasal secretions
5. High rate of false negatives in some tests
6. 15 minutes for results
7. Does not distinguish influenza strain type
8. Does not measure viral load

Reverse transcriptase polymerase chain reaction

1. Tests for viral genetic material
2. Nasal or throat swabs
3. Some false negatives
4. Can distinguish between Type A&B
5. Very fast results <24 hours

Enzyme Linked Immunosorbent Assay (ELISA)

1. Tests for antibodies
2. Usually tests for the Hemagglutinin portion of the influenza virus
3. Can be developed to test for specific portions of influenza virus and not the whole epitope
4. Serologic test
5. Does not quantify viral load
6. Cannot be used for subtyping
7. Correlates well with HI testing

Virus Culture

1. Gold Standard for diagnosing influenza
2. Serologic test
3. 3-10 days for results
4. Identifies which virus and which strains are present
5. Quantifies viral load
6. Used by OIE for genetic influenza chart

Single Radial Hemolysis (SRH)

1. Serologic test
2. Sensitive, specific and reliable
3. Identifies antibodies – usually IgG
4. More commonly used in human influenza testing
5. Indicates seroprotection according to hemolysis ring

Hemagglutinin Inhibition (HI)

1. Serologic test
2. Provides titer levels (1:110, 1:330, etc)
3. Titer levels correlate with level of protection against EIV
4. Detects antibodies to HA
5. Commonly used in correlation with challenge studies

Influenza Challenge Studies

- Cross Reactivity Challenge
- Active Challenge
  - Experimental – controlled
  - Real Life – not controlled

Influenza Cross Reactivity Challenge

1. Used to evaluate/screen efficacy of EIV strains
2. Serologic test performed in the laboratory
3. Can test against multiple EIV strains at one time
4. Cost effective – unlimited number or horses
5. Used by the OIE to determine relevant EIV strains *****
6. Does not carry the risk of introducing the virus to the equine population

7. Provides titer levels against specific strains which can correlate with protection
8. Does not provide clinical signs associated with infection

Influenza Active Challenge

1. Controlled or real life
2. Controlled /experimental usually done with one challenge strain of EIV
3. Used for licensing of EIV vaccines
4. Time consuming and expensive (>250K)
5. Must be performed in BL2 certified facilities
6. Number of challenge horses is limited
7. Severe challenge – not realistic

Treatment

The only available and effective treatment is excellent supportive care and rest for a minimum of 3 weeks.

Vaccination

Unfortunately, EIV vaccines are not able to fully keep pace with the continual changes in antigenic strains (equine-antigenic drift). This differs from human influenza vaccines (antigenic shift), which are updated on an annual basis based on immunosurveillance data. Several EIV vaccines are available, including inactivated parenteral, modified-live intranasal, and a vector-based vaccines. The modified-live and vector-based vaccines appear to be most efficacious. Vaccination should start when maternal antibodies have waned at 5-6 months, with boosters given 3-4 weeks later and again in 3-4 months. Vaccination should be boostered every 6 months *(At a minimum for performance horses)* in horses that are frequently exposed to other horses.

Equine herpesvirus

The common name for equine herpesvirus infection is equine rhinopneumonitis, a designation that, like equine influenza, indicates that the disease is not limited to the upper respiratory tract. Although both EHV-1 and EHV-4 can cause rhinopneumonitis, EHV-4 is more commonly implicated. However, EHV-1 is notorious for its ability to cause abortion and neurologic disease in addition to the respiratory syndrome.

Epidemiology

Epizootics of respiratory disease can be caused by EHV and it is a more ubiquitious equine respiratory pathogen than EIV. However, EHV remains latent in infected horses, residing in the trigeminal ganglia or in T-lymphocytes in the respiratory lymph nodes. Thus, EHV creates an enormous population of carrier animals, ensuring that equine rhinopneumonitis will remain endemic throughout the horse population. Although the majority of adult horses develop a strong immune response to EHV-1 and -4, they continue to spread the virus among naïve populations, especially young horses that travel.

Pathogenesis

Both EHV-1 and EHV-4 are naturally acquired via respiratory transmission. As with EIV, EHV is readily spread in aerosols expressed by persistently coughing horses. EVH can also be easily spread by fomites. The EHV incubation period is 2-5 days, followed by viral shedding for up to two weeks. Respiratory herpesviruses cause destruction of respiratory epithelial cells, enabling secondary bacterial infection to occur. Primary viral pneumonia caused by EHV is also possible. After the virus replicates in the respiratory epithelium, the animal becomes viremic. This is the pathogenesis for EHV neurologic disease or abortion. EHV-4 is restricted to the respiratory epithelium. EHV-1, on the other hand, is endotheliotropic, with a predilection for respiratory, adrenal, thyroid, placental, and CNS vascular endothelium. This explains its ability to cause both abortion and neurologic disease.

Diagnosis

The diagnostic approach is similar to that for EIV, although there is not yet an available EHV stall-side test. A commercial PCR diagnostic test for EHV-1 and EHV-4 (RealPCR™, Iddex) is available for evaluation of samples

submitted as whole blood or nasal swabs. Because presence of EHV in nasal secretions and circulating leukocytes may not overlap, testing of both specimens is recommended to achieve optimum diagnostic sensitivity. The PCR test has acquired new importance in light of the increase in recent years in myeloencephalopathy caused by neuropathic EHV-1. Molecular PCR assays are capable of identifying the EHV-1 D_{752} genotype associated with neurologic cases and distinguishing it from the EHV-1 N_{752} genotype more commonly found in EHV-1 abortions. The commercial PCR test is reportedly capable of identifying the D_{752} mutant strain. It should be noted that a small percentage of myeloencephalopathy cases are caused by EHV-1 strains without the D_{752} neuropathogenic marker. The clinical significance of this new diagnostic technology is arguable, but it may be useful in determining increased risk of EHV neurologic disease and implementing appropriate biosecurity.

Treatment

The only practical treatment for EHV clinical disease is supportive care and excellent nursing. As with EIV, horses must be allowed at least 3 weeks of rest before returning to work.

Prevention and Treatment Strategies

Vaccination against EHV-1 and 4, and equine influenza virus infection remain a cornerstone for the control of equine viral respiratory disease. Inactivated vaccines are the most common type of vaccine in use although modified live and recombinant vaccines are available. The value of inactivated vaccines critically depends on the quality and quantity of viral antigen and the adjuvant utilized. Treatment modalities for equine respiratory disease may involve multiple strategies.