Update on Equine Herpes Virus & respiratory diseases

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Equine Herpes Virus
- 9 herpesviruses infect equids (1–5 horse pathogens)
- Equine Herpes Myeloencephalopathy (EHM) first reported in Norway 1966
- Past 12 yrs, EHM more widespread (primarily EHV-1 but 4 has been linked)
- EHV-1 & 4 co-evolved with horses over millions of years (carrier state)

EHV-1 and EHV-4 Overview
- Can cause significant upper respiratory tract disease (URTD)
- EHV-1 and EHV-4 are ubiquitous in the world’s horse population
- Can cause URTD without major predisposing factors
- Circulate and transmitted year round
- Most often cause sub-clinical disease

EHV-1 and EHV-4 Overview
- Both have potential for causing widespread outbreaks of URTD
- Highest incidence of disease is in young horses
- Highest risk of infection between weaning and two years of age
- Spreads rapidly through susceptible horse populations

Agenda
- Equine herpesvirus-1 & (EHV-4)
  - Overview of current outbreak in cutting horses
  - EHV facts
  - EHV protection
- Equine Influenza
- Strangles
- Research update
- Questions
Pathogenesis

- Respiratory tract is the natural entry point for EHV-1 and EHV-4 virus, with the respiratory mucosal epithelium being the primary target for infection
- Viral infection can be acquired by close physical contact with a horse that is actively shedding the virus or with virus contaminated fomites
- Viral aerosol transmission is less than with EIV

Equine Herpes Virus (Pathogenesis)

- Exposure occurs via respiratory route
- Virus replicates in nasopharyngeal epithelium
- Mucosal erosions & viral shedding (4–7 days)
- Virus infects leukocytes & endothelial cells & viremia ensues with dissemination
- Infection of vascular endothelium–ischemia

Pathogenesis

- Incubation period is 2–5 days after exposure
- May shed virus for 14 days
- Peak viral shedding occurs during first few days after onset of nasal discharge and coincides with the febrile faze
- Pathology of EHV URTD is focal, cytolytic destruction and exfoliation of the nasopharyngeal respiratory epithelium
- Degree of viremia is key

EHV-1 pathogenesis (1).

EHV-1 pathogenesis (2).

Differences between EHV–1 & EHV–4

- EHV–1 and EHV–4 are 70% genetically similar
- EHV–1
  - Upper respiratory
  - Abortion
  - Newborn Still Birth
  - Neurological
    - Mainly D<sub>752</sub> point mutated EHV–1 strain
- EHV–4
  - Mainly Upper Respiratory
**Equine Herpes Virus**
- Commonly affects younger horses – weaning to 2-3 years
- Spread via inhalation & direct contact (ubiquitous in equine populations)
- Natural immunity is short lived (60 days)
- EHV-1 & 4 primary concern to horses – latent in lymph nodes of respiratory tract (EHV1) and trigeminal nerve ganglia (EHV4)
- Once infected, they carry the virus for life
- EHV 2 & 5 new players?

**Respiratory Disease Prevalence**
- 75-80% of diagnosed respiratory disease is due to EHV-1 and EHV-4
- Greater than 90% of those cases are due to EHV-4
- Uncommon in foals less than 3 months of age
- Most cases of URTD caused by EHV-4 occur between 4-12 months of age
- EHV-4 URTD is more severe in yearlings that were not exposed at an earlier age

**US Equine Respiratory Pathogens**
- 24 month study
- 761 samples submitted
  - fever, coughing or nasal discharge: 201/761 (26.4%)
  - PCR positive for one or more pathogens:
    - EHV-4 (82 cases)
    - EIV (60 cases)
    - S. equi (49 cases)
    - EHV-1 (23 cases)

**Respiratory Disease Prevalence**
- URTD due to EHV commonly referred to as “foal snots”
- Yearling sales events have a significant incidence of URTD due to EHV-4
- Older horses (2-3 yrs of age) in training, on the racing circuit or show barns URTD caused by EHV-1 and EHV-4 are common
- Previously exposed horses older than 3 yrs of age continue to have periodic reinfection by EHV-1 and EHV-4 throughout their lifetime

**Epizootology**
- Most horses have seroconverted to EHV-4 by 2 years of age – less with EHV-1
- Post infection latency occurs for the life of the horse
- Reservoir for EHV-1 and EHV-4 is globally distributed latently infected horses – approximately 50% of population
- Life cycle is dam to foal, latency in affected foals, periodic reactivation and viral shedding

**Epizootology**
- Majority of viral transmission events usually results in URTD result in inapparent or mild clinical signs
- Seroconversion rate for EHV-1 and EHV-4 exceed the incidence of clinically observed disease
- Stress causing the latent virus to reactivate and shed is the main factor in disease outbreaks
Both EHV-1 and EHV-4 can be latent together and persist for the life of the horse.

As many as 60% of horses recovering from a primary respiratory disease can become latently infected with EHV-1 and EHV-4.

Reservoirs for latent EHV-1 and EHV-4 are the trigeminal nerve ganglia and upper respiratory lymph nodes.

Latency

Latent virus is protected from recognition by the immune system even in the face of strong acquired immunity.

Reactivation of latency and viral shedding occurs with various episodes of stress or corticosteroid administration.

Latent virus that is reactivated can be shed without the horse showing any clinical signs of disease.

EHV Risk factors

- Horses under stressful situations, transport, show,...
- Horses in large groups, concurrent disease

Clinical Disease

Acute rhinitis and pharyngitis, and can extend to the distal airways.

Clinical signs are highly variable and can range from inapparent disease to life-threatening, primary viral pneumonia.

Bilateral nasal, clear serous discharge is most common signs of early infection.

Serous secretions carry high levels of infectious virus.

Clinical Disease

Serous nasal discharge will progress to a mucoid nasal discharge by the 2nd or 3rd day.

Mucoid nasal discharge changes to mucopurulent nasal discharge after day 5 after clinical onset.

Pyrexia - as high as 41°C, submandibular lymphadenopathy, conjunctivitis, lethargy and anorexia, minimal coughing.

Occasional lower respiratory tract involvement.

Clinical signs and viral shedding greatest during first few days of disease.

Prognosis for full recovery is good, usually by the end of two weeks, with very low mortality.

Severe secondary bacterial infection can complicate the disease process.
EHV: Recognizing the Signs

- Fever and/or cough
- Nasal discharge
- Mild Limb edema
- Poor appetite and depression
- Abortion
- Poor anal / tail tone
- Inability to retract penis
- Ataxia
- Bladder paresis
- Fecal incontinence
- Muscle fasciculation
- Increased respiratory rate
  - (>14 b/min)

Complications and Sequelae

- Secondary bacterial infection by Strept zoo is common following URTD caused by EHV and can progress to severe respiratory disease
- EHV-4 replication is usually restricted to the URT and regional lymph nodes
- EHV-1 infection has the potential for more serious clinical disease (respiratory, abortion, neonatal foal death, myeloencephalopathy, pulmonary vasculotropic infection, and ocular disease).

Dr. Julie Wilson Slide

Diagnosis

- Respiratory disease caused by EHV-1 or EHV-4 can be indistinguishable from other respiratory diseases based on clinical signs
- Key to diagnosis is identifying presence of EHV-1 or EHV-4 in either nasopharyngeal secretions or blood from infected horses
- Best diagnostic results occur when nasopharyngeal samples are taken within 48 hours of onset while horse is pyrexic and nasal discharge is still serous

Use a 25.4cm dacron swab to swab both nasal mucosal surfaces – keep moist and sealed in red top tube or in transport media

Collect at least 7 mls of blood in red top tube and keep chilled

Nasal swabs can be used for PCR testing and blood for virus isolation of buffy coat

EHV-1 and EHV-4 can be distinguished from each other using diagnostic testing

Data from Dr. Steve Reed OSU

EHV: Analysis Whole Blood & Swab

| Days post-infection | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---------------------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Fever & Nasal Shedding | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |

EHV-1 log copies

Data from Dr. Steve Reed OSU
**Treatment**

- Depends on severity of clinical signs
- Goals are to decrease clinical signs of infection, maintain hydration and feed intake, minimize secondary bacterial infections, and control or decrease level of viremia
- Non-steroidal anti-inflammatory to control pyrexia and inflammation
- Antibiotics

**Prevention**

- Prophylactic immunization, preventive health program, and good bio-security measures
- Vaccination against EHV–1 and EHV–4 is recommended by the Equine Infectious Disease Advisory Board for all horses that are at risk for acquiring infection
- Foals become sero-negative by 5–6 months of age
- Titer levels for EHV are not indicative of protection

**Prevention**

- Vaccination does not prevent infection – it decreases disease incidence, severity of disease and viral shedding
- It is recommended that horses less than 3 years of age be vaccinated with a product that contains both EHV–1 and EHV–4
- Natural immunity is approximately 60–90 days
- Goal of vaccination is to decrease level of viremia

**Vaccination**

- Vaccination and boosters should be timed with periods of stress in mind (weaning, transport, sales, training, relocation, shows)
- Recommended vaccinations – 2 doses IM 4 weeks apart starting at 3 months of age, with booster doses every 6 months after 12 months of age for competitive horses (at risk)
- Broodmares at 5, 7 & 9 months of gestation
- Latent carrier state is not prevented by vaccination

**Characterization of immune responses in healthy foals when 1 a multivalent vaccine protocol was initiated at 90 or 180 days of age**

E. G. Davis, N. M. Bello, A. J. Bryan, K. Hankins and M. Wilkerson
Department of Clinical Sciences and 26 Diagnostic Medicine Pathobiology, College of Veterinary Medicine, 37 Department of Statistics, Kansas State University, Manhattan, Kansas 66506,

Reasons for performing study: Protection from infectious disease requires antigen specific immunity. In foals, most vaccine protocols are delayed until 6 months to avoid maternal antibody interference. Susceptibility to disease may exist prior to administration of vaccination at 4–6 months of age.

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Outbreak Response to clinical EHV-1

- Early Diagnosis – vital, know lab and proper samples (isolation, swabs for PCR)
- Prevention of further spread – disinfection, isolation, prevent aerosol & fomite transmission. Vaccination of horses at > risk?
- Management of clinical cases
- In environment – virus unlikely to survive > 21 days

Disease Outbreak Control

- Outbreaks of URTD caused by EHV often spreads rapidly, and significant economic and equine welfare consequences can occur
- Priority during an outbreak is to prevent spread of the virus from infected horses to other horses
- Isolation, quarantine and disinfection are key
- Remove all clinically ill horses from premise and move to an isolation facility if possible

EHV vaccines – antigen load

- Flu/Rhino combos
  - 30-33 Million Plaque Forming Units (PFU’s)
- Prodigy
  - 100 Million PFU’s
  - No challenge data with EHM
- Rhinomune
  - Modified Live
  - EHM challenge data available
- Pneumabort K
  - 133 Million PFU’s
  - EHM challenge data available
  - Contains Army 183 and Ab4 strains of EHV-1
  - Both are D752 genotype

Prevention of Equine Herpes Myeloencephalopathy by Vaccination:
A Pilot Study

- Objective – To determine if vaccination with a high antigenic mass vaccine (Pneumabort K + 1b) is able to reduce or prevent clinical in the face of a severe viral challenge with a neuropathogenic strain of EHV-1.
- Materials/Methods – Twelve aged mares (20 years old) were randomized to either saline control or vaccination groups. Three intramuscular injections with a high antigen vaccine were administered at monthly intervals followed by inoculation with a neuropathogenic strain of EHV-1. Ataxia scores, rectal temperatures, and clinical scores were determined.
Neurologic EHV Challenge

Blinded study
Evaluated:
- Ataxia
- Viral shedding
- Rectal Temperature
- Clinical scores
- Viremia
- Titer Levels

Results - Evaluated for 21 days after challenge
- Vaccinates had statistically less:
  - Ataxia
  - Rectal Temperatures
  - Viral Shedding
  - Viremia
  - Clinical Scores

Blinded study Evaluated:
- Ataxia
- Viral shedding
- Rectal Temperature
- Clinical scores
- Viremia
- Titer Levels

Equine Influenza

Influenza virus
- (A1 & A2)
- Highly contagious
- Horses inhale virus
- Particularly prevalent in young horses
  - Generally seen in horses 2 years of age and older
- Major concern in areas of high horse population density
- Disease increases risk of secondary bacterial infection

Equine Influenza

Prevention of Equine Herpes
Myeloencephalopathy by Vaccination: A Pilot Study

- Conclusion - Immunization of horses with a high antigen vaccine, Pneumabort K®
  significantly decreased viremia and appeared to decrease the severity of disease, as measured by several other disease parameters. Of particular interest, the severity of ataxia appeared to be lower in the vaccinated horses as compared to the saline controls although statistically significant differences were not observed.

Equine Influenza

EIV- epidemiology

- Influenza A evolved from ancestral avian precursor into different lineages infecting many species - including horses
- Birds are the natural reservoir of influenza A - pivotal role in emergence of new strains to infect mammals
**Equine Influenza**
- Infects & replicates in ciliated epithelial cells in upper & lower respiratory tract
- Results in de-ciliation of large areas w/in 4 to 6 days
- Incubation period= 1 to 3 days
- Fever, harsh dry cough= release of large amounts of virus
- Serous…mucopurulent nasal discharge, myalgia, inappetance, enlarged lnn.

**Normal trachea**
- Image courtesy of Dr. Issel and Gluck Center

**Trachea after cilia destroyed by flu**
- Image courtesy of Dr. Issel and Gluck Center

**Immunological Protection from EIV—Summary**
- Dependent on different immune mechanisms at both local and systemic level
- Systemically— IgGa and IgGb responses associated w/ protection and likely depends on an IFNγ mediated Th1 immune response
- Mucosally— production of IgA critical and typically depends on Th2 immune response

**Equine Influenza**
- Rapid progression through the herd
- Attaches to epithelium via HA spikes
- Destroys epithelium and clumps cilia
- Regeneration requires 1 week for every day horse runs a fever

**Influenza A**
- Antigenic Shift
  - Sudden change in HA
  - Recombines with another influenza
  - Human & Avian
- Antigenic Drift
  - Random mutation in HA and NA
  - Limits long-term vaccine efficacy
  - Horses...
Clade 1 and Clade 2

Severe viruses from North America were classified as members of the Florida sublineage (clade 1), similar to A/eq/Winconsin/03. In conclusion, a variety of antigenically distinct equine influenza viruses continue to circulate worldwide. Florida sublineage clade 1 viruses appear to predominate in North America, clade 2 viruses in Europe.

EIV H3N8 Generational History
1986 – H3N8 virus diverged into American & Eurasian lineages (American dominant)
1990 – American lineage diverges into Kentucky & Florida sublineages (Florida dominant)
Early 2000’s – Florida sublineage diverges into two homologous clades: 4,5,6
- Clade 1 (predominantly North America) 5
- Clade 2 (predominantly Europe) 6
2010 – OIE recommendation – Vaccines sold internationally should have clade 1 & clade 2 EIV strains 1
Genetic homology – Kentucky/97 (Fluvac Innovator® virus) has genetic homology with all subsequent Florida sublineage isolates 6
Cross-reactivity against Richmond/07 – Kentucky/97 cross-reacts against clade 2 Richmond/07 virus

EIV Florida Sublineage Phylogenetic Tree
Zoetis has a legacy of evaluating FLUVAC INNOVATOR® for cross-reactivity against newly emerging equine influenza virus (EIV) isolates to help ensure that the vaccine remains immunologically current.

OIE, the world organization for animal health, recommends that EIV vaccines for the international market contain a clade 1 and clade 2 virus of the Florida sublineage.¹ OIE considers the Richmond/07 virus to be a contemporary representative of clade 2 EIV.¹

Zoetis conducted a serologic study of horses vaccinated with FLUVAC INNOVATOR to determine if the vaccine cross-reacts against the Richmond/07 virus.

Study Design:

80 EIV seronegative horses
- Group 1 (n = 40): Vaccinated with West Nile Innovator EWT and Fluvac Innovator EHV-4/1
- Group 2 (n = 40): Sham vaccinated with saline (control group)

Vaccination schedule
- Vaccinated days 0 and 21

Serologic methods
- Serum samples obtained days 0, 21, 28, 42
- HI titers measured with non-ether technique for cross-reactivity to Richmond/07

HI assays were performed using a non-ether method of stabilizing the test antigen, which yields a lower HI titer than ether treatment.²,³

Primary goals:
1. To reduce clinical signs,
2. To shorten convalescent period,
3. To reduce shedding

Ideally, provide long-term immunity, efficient memory response & cross protection

Vaccine efficacy = clinical protection, absence of pyrexia, cough and discharge

Virological protection = absence of virus in secretions

AAEP Influenza Guidelines

1. Previously vaccinated adult horse – annual (low risk) – semi annual (high risk)
2. Adult with unknown history – 3 doses – 2nd dose 4–6 weeks after first dose, 3rd dose 3–6 months after second dose
3. Foals – 3 doses – 1st dose at 6 months of age, 2nd dose 3–4 weeks after 1st dose, 3rd dose at 10–12 months of age
Biosecurity

- Segregation of horses into smaller groups
- Clean then disinfect stalls and equipment with a proper disinfectant
- Isolate new horses for a minimum of 3 weeks before introduction into the herd
- Clean then disinfect all shared buckets, tack or equipment
- Maintain all horses on a current vaccination schedule

NEW STUDIES

Epidemiologic and economic cost of a "strangles" outbreak on a large broodmare farm (14EQPETAIF01)

- History:
- Methods and Materials
- 1400+ resident broodmare farm
- 2 years of clin path data and clinical outcomes while using abx’s, vaccination and biosecurity measures to control "Strangles" epidemic on farm

Vaccination; Pinnacle

- Avirulent live S.equi vaccine (IN)
- S.equi strain modified to remove HA capsule from cell wall
- Removal of capsule ‘cripples’ ability of vaccine to cause/spread disease yet maintains ability to stimulate immune response
- Recent improvement in genetic stability of vaccine by deletion of 2 genes responsible for capsule synthesis
- This deletion provides means of differentiating vaccine from ‘wild’ S.equi strains by PCR
- K State study; effectiveness of Per Os administration
- AAEP 2014
- Pinnacle 2014 ADE reports; 2.7/10,000

Lessons learned from a strangles outbreak on a large Standardbred farm

- A 15-month outbreak involving 62 clinical cases of strangles occurred on a large Standardbred breeding farm (average population of 1,400 horses). Sixteen asymptomatic horses were found to be PCR-positive for S. equi ssp equi. During the outbreak, serologic samples from 48 clinically normal horses were found to be seropositive for S. equi ssp equi, confirming herd-wide exposure. After several clinical cases of strangles had been diagnosed, an intranasal S. equi ssp equi vaccine was administered to clinically normal horses (n = 558) considered to be at risk of exposure. Strangles complications included 7 fatalities (none in vaccinated horses) and 6 cases of purpura hemorrhagica (4 in vaccinated horses).
- Midway through the outbreak, injectable, sustained release ceftiofur given as an initial dose followed by a second dose 4 days later was used exclusively for systemic antimicrobial treatment of clinically affected and PCR-positive horses. This antimicrobial regimen coincided with a reduction in disease incidence and eventual resolution of the outbreak.
Lessons learned from a strangles outbreak on a large Standardbred farm

- **Results**
  - Outbreak brought under control along with ID of "carriers" and biosecurity measures
  - DO NOT recommend IN Pinnacle in the face of a Strangles problem on a farm

- **Timeline**
  - Submission to EVE: March '15

Questions?

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