Abstract:
Equine Herpes Viruses (EHV) are some of the most widespread pathogens in the equine world. EHV-1 and 4 cause diverse and important disease syndromes. One of these, equine herpes myeloencephalopathy, is potentially increasing in frequency and is regarded as an emerging disease. The accurate diagnosis and treatment of EHV is a challenge and an evolving area. Practical biosecurity and herd health measures are critical to the prevention of EHV and the critical but complicated role of vaccination is discussed.

Introduction:
There are nine different equine herpesviruses (EHVs). Five types (EHV-1 to EHV-5) infect the domestic horse and EHV-6 to EHV-9 are associated with infections in wild equids including asses and zebra. The alpha herpesviruses EHV-1 and EHV-4 are respiratory pathogens and are also responsible for abortion and neurological disease. EHV-3 is a venereal pathogen causing coital exanthema and will not be discussed in this article. The gamma herpesviruses EHV-2 and EHV-5 may be clinically important in certain situations causing ocular and respiratory disease. The management of these are beyond the scope of this article and we will focus on the commonest and most important clinical pathogens EHV-1 and 4.

Different Disease Syndromes
EHV-1 is associated with three clinical disease syndromes:

- Respiratory disease
- Abortion
- Neurological disease - Equine Herpes Myeloencephalopathy (EHM)

The virulence of individual isolates shows considerable variation. There are neuropathogenic and non-neuropathogenic strains however both can cause EHM and the differentiation is not very usefully clinically. EHV-4 is principally a cause of respiratory disease, although some highly virulent endotheliotropic, abortigenic strains exist.

Diagnosis:
History and clinical signs: are important but are usually insufficient on their own to confirm diagnosis. EHV infections often do not cause clinically apparent respiratory disease and even then there is little to distinguish EHV respiratory disease from other viral and bacterial pathogens. A common misconception is that monitoring for respiratory clinical signs provides a warning for
impending abortion or EHM: most horses that abort or develop neurological disease after EHV infection do not show signs of respiratory disease first.

_Sampling to confirm the diagnosis._ Case selection is critical: all samples for direct demonstration of virus should be collected from early clinical cases (ideally <5 days) whenever possible. This best achieved by identifying in-contact cases with pyrexia that may have not showed any other clinical signs as yet, although samples from clinical cases may also be taken especially if they appear to be the only horse affected. Proper selection of cases for sampling is particularly important in EHM cases where clinical signs appear towards the end of viraemia and viral shedding is waning or may have ceased.

Samples:

**Nasopharyngeal swab:**

A long highly absorbent swab (see figure 1) passed up the ventral meatus of the nasal passageways to the level of the medial canthus to ensure adequate sampling of the nasopharyngeal mucosa, placed in viral transport medium (e.g. white top tube from AHT). The sample should be refrigerated and time minimized before arriving at the laboratory.

- Quantitative Polymerase chain reaction (qPCR) – Detects viral DNA. Sensitive and allows estimation of the amount of virus (‘viral load’) in sample^{3,5}. Quick results
- Immunofluorescence (IF) – Detects viral antigen expressed on the surface of infected cells. Quick but not as sensitive as qPCR and does not give an estimate of the amount of virus.
- Virus isolation – traditionally regarded as the ‘gold standard’ test but slow and insensitive compared to qPCR and IF. Virus is grown in cell cultures inoculated with the supernatant from the sample. Usually takes 5-7 days of culture.

**Blood:**

- qPCR to detect virus DNA in buffy coat cells (mainly lymphocytes)
- Serology to detect antibody induced by infection
  - IgM – Detectable 4-5 days after infection, peaking at 20-30 days returning to baseline between 60-80 days^{5}. Measured by compliment fixation (CF)^{7,8}.
  - IgG – Detectable 8-10 days after infection, peaking at 30-40 days persisting for many months (>9mths). Measured by virus neutralisation (VN) or ELISA (can differentiate between EHV-1 and EHV-4).
  - Take baseline sample and repeat sample 10-14 days later^{5}. Significant increase in IgM antibody level is usually taken as threefold to fourfold increase.
  - A high CF titre (IgM) in a single serum sample from a non-vaccinated horse provides good preliminary evidence of infection and is a valuable initial diagnostic test in suspected EHM cases.
  - N.B. Previous vaccination and maternal antibodies confound the interpretation of serological investigations.
- Haematology – non-specific and difficult to interpret – Initial (first 7-10 days) transient leucopaenia with lymphopaenia which is replaced by a leucocytosis with lymphocytosis up to day 21 days after infection.
Tissue: e.g. Foetal, placental or central nervous system tissue

- Histopathology – characteristic eosinophilic inclusion bodies or vasculitis and often thrombosis of CNS blood vessels.
- Immunohistochemistry (IHC) – paraffin-embedded, formalin fixed tissue – demonstrate viral antigen.
- In situ hybridization (ISH) - paraffin-embedded, formalin fixed tissue – demonstrates viral DNA.
- PCR – Fresh, frozen and fixed tissue samples.
- IF – Frozen sections from aborted foetal and placental tissues.

Cerebrospinal Fluid (CSF): EHM cases

- May be xanthochromic (yellow discolouration) – see figure 2: normal CSF above xanthochromic below.
- Increased total protein without a concomitant increase in total white cell.
- These two changes above plus characteristic clinical signs are suggestive of EHM but not diagnostic of EHM.
- Antibodies to EHV-1 in the CSF may be from leakage from the vasculitis and may not be from local production so does not definitively confirm EHM, only EHV-1 exposure.

Treatment:

Respiratory Disease:

- Generally mild and self-limiting disease and does not require specific treatment.
- Rest, dust free management and biosecurity measures to stop transmission are indicated.
- Broad-spectrum antibiotics are often administered but seldom indicated to prevent secondary antimicrobial infections.
- Clenbuterol (Ventipulmin™, Dilaterol™) to stimulate mucociliary clearance – usually not required.
- Dembrexine (Sputolosin™) to act as mucolytic – usually not required.
- Preventative when stress or mixing has to take place – potential value in immunostimulants - Parapox ovis (Zylexis™).

Abortion:

- No evidence that treatment of in-contact mares with antiviral agents (e.g. nucleoside analogues) prevents abortions.
- Ensure whole placenta has been passed (usually the case); in the rare event this does not occur treat for retained foetal membranes.
- Rigorous biosecurity – see below.

EHM:

- Adequate bedding to prevent trauma and quiet environment to prevent excitement.
- Recumbent horses can be nursed successfully in slings (e.g. Anderson sling – see figure 3).
• Indwelling Foley catheter in horses with bladder paralysis, urinary retention and overflow – see fig – Application of petroleum jelly around the perineum along with an extension line to direct urine away and prevent scalding. Maintain sterility but cystitis a common complication and antimicrobial therapy is often indicated.
• Faecal evacuation if faecal incontinence
• Fluids and indwelling feeding tubes
• NSAIDs – e.g. flunixin meglumine 1.1mg/kg IV BID
• Corticosteroids – e.g. Sodium phosphate dexamethasone 0.1mg/kg IV SID
• Anti-oxidants - Vitamin E, DMSO and thiamine.
• Nucleoside analogues\textsuperscript{13-17} – See Figure 4
  - Acyclovir – 10mg/kg PO five times daily – poor bioavailability and questionable efficacy with EHV-1
  - Valacyclovir - 20-40mg/kg po tid – better bioavailability and efficacy than acyclovir but more expensive
  - Ganciclovir – 2.5mg/kg intravenously q8hrs for first 24hrs the orally every 12 hours – best in vitro testing of all nucleoside analogues – expensive.

Prevention of Spread of Disease:

EHV-1 disease control programs have three goals: See HBLB code of practice \url{www.hblb.org.uk}

1. Prevention of disease entry to premises
2. Limiting the spread and severity of disease
3. Limiting the spread to adjacent properties.

\textit{Prevention of disease entry to premises}

Prevention of disease entry is difficult because the majority of horses carry latent EHV infections. To reduce the risk of disease entry new arrivals should ideally have been vaccinated before arrival and should be kept isolated from other horses until sufficient time has passed for disease to become apparent. On studs, newly arrived and “walk-in” mares should be kept strictly separate from resident in-foal mares for 56 days after covering. Mares arriving at studs to foal should be transported at least 28 days before the foaling due date. Horses that have arrived from sales or markets are high risk and should have more stringent isolation and biosecurity measures. In yards with no pregnant mares isolation period of at least 21 days is advised because viral shedding may occur after reactivation of the virus induced by the stress of moving. Minimising stress in resident horses, including while being transported, disruption of established social groups, and at weaning should assist in reducing the frequency of reactivation of the virus from the latent state.

\textit{Limiting the spread and severity of disease}

• Different age groups should not be mixed
• Group size should be kept small as practicable.
• Pregnant mares should be separated from other horses and kept in small groups to minimise the risk of a large-scale outbreak.
- Mares in their last trimester should be ideally housed and managed individually.
- Isolation areas should geographically separate and rigorously maintained.
- If a suspect case occurs the horse should be isolated immediately and appropriate samples taken.
- Any in-contacts should be isolated and monitored for disease (take twice daily temperatures)
- If the in-contact group is large and it is practical to do so, it should be subdivided.
- Environmental contamination – the virus can survive for limited periods depending on the surface and prevailing weather conditions. All discharges from affected horses should be removed and the area disinfected with approved disinfectant e.g Virkon. Bedding should be burned.
- Stop all movements
  - Isolate aborted mares for 28 days and do not mix with pregnant mares for 56 days.
  - EHM horses should be kept isolated for minimum of 14 days\(^\text{18}\) and sometimes up to 28 days to account for the maximum possible duration for viremia. Testing of viral shedding can be undertaken to shorten the isolation period.
  - Movement of all horses on and off the premises should stop for a period of 28 days.

Limiting the spread to adjacent properties:

- Efficient communication between attending vets, premises owners and other parties working with the affected premises.
- Care with personnel and fomites – easy and clear biosecurity measures should be implemented.

Vaccination – Pros and Cons

There are two EHV vaccines licenced in the U.K.. The one used presently is inactivated vaccine containing EHV-1 and EHV-4 (Equip EHV-1,4, Zoetis). The other (currently off the market) is also an inactivated vaccine containing EHV-1, 4 and inactivated influenza virus (Equilis Resequin, MSD). Worldwide there are 10 killed commercial EHV vaccines available (8 in United States and 2 Europe) and two live vaccines (1 in USA and 1 in Europe). None of these vaccines induces a ‘perfect’ sterile immunity (complete clinical and virological protection) that prevents all disease syndromes and the development of a more effective EHV vaccine is a priority worldwide in EHV research. However, it is important to be clear that vaccination with current vaccines has an important role to play in reducing the risk and impact of clinical disease. The UK vaccines induce high titres of complement fixating and virus neutralisation antibodies. They reduce the duration and titre of nasal virus shedding of virus but there are contradictory reports of the ability of killed vaccines to reduce viremia and hence stop abortion\(^\text{19,20}\). With regards to abortion the field data is unclear. The introduction of vaccination in the early 1960’s coincided with a marked reduction in EHV-1 abortion rates across the world\(^\text{21}\) however simultaneously vigorous hygiene measures were introduced and the relative impact of both is impossible to assess in light of the lack of randomised, controlled field studies. Current vaccines do not make any claims for efficacy against EHM because the syndrome is difficult to reproduce experimentally and there are no reliable field data on the effect of vaccination of prevention of EHM because the syndrome is uncommon and large scale outbreaks are infrequently reported in the UK.
The reasonable expectation of current vaccines should not be to produce sterile immunity but rather to reduce the severity of clinical disease (respiratory) and limit virus shedding from infected horses, thus reducing contagion. Vaccines should therefore be used to supplement hygiene control measures, which as previously discussed have a central role in reducing exposure to the virus.

The use of vaccination in prevention of EHM is even less clear as this is thankfully a rare event and assessing vaccine efficacy more difficult. Vaccinated horses do get EHM and none of the current UK vaccines are licenced to protect against EHM. The case for vaccination is further complicated by the initial analysis of a large outbreak in 2003 at Ohio State University which seemed to show an increase risk of EHM in vaccinated horses. This also seemed to support the then theory of immune-mediated pathogenesis for EHM. We now know that EHM is caused by vascular endothelium cell death and local ischaemia causing neuronal cell death. We also know that old horses (>15yrs old) are a potential experimental model for EHM. When age is accounted for in the Ohio data the increase in risk of vaccination is removed and therefore the direct causality of EHV vaccination and EHM is unclear and could easily be accounted for by age. The number of times a horse is vaccinated is only one factor that affects the horse immune system and it may be that age-related changes (immunoscience) have an important role to play. Further investigation is required to elucidate this interaction further. It is this author’s opinion that general vaccination at herd level (i.e. all horses on a yard) is beneficial in decreasing nasal shedding of the virus and therefore environmental contagion. Yard vaccination in the face of an EHM outbreak is contra-indicated as the state of infection/immune response of the individuals will be unknown. Vaccination of closely associated horses (e.g. geographically), but separate from the present outbreak, is potentially beneficial and should be judged on an individual basis in consultation with the testing laboratory and/or epidemiological input. However the fact remains that vaccination remains a valuable tool used appropriately to reduce environmental contagion and reducing disease incidence integrated in to biosecurity and hygiene measures.

Acknowledgement:

Thanks go to Professor J. Slater for reading this manuscript.

Suggested Further Reading:


References:


Figure 1: Nasopharyngeal Swab

Figure 2: Yellow discoloration of xanthochromic CSF in EHM case
Figure 3: An EHM case being managed in an Anderson Sling. Picture courtesy of A. Draper MRCVS

Figure 4: Valacyclovir treatment being used in a quarantine area to treat EHM cases. Picture courtesy of A. Draper MRCVS