A critical view on Equine Herpesvirus-1 and on Equine Herpesvirus-associated Myeloencephalopathy (EHM)

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An infection with Equine herpesvirus type 1 (EHV-1), officially and by convention now called EQUID Herpesvirus, is the cause for Equine herpesvirus-associated Myeloencephalopathy or EHM. In 2008 the USDA declared this syndrome an 'emerging disease' among horse populations in the U.S.A., which indicates that EHM is perceived as an expanding problem that is observed more frequently when compared to the past.
EHM is a reportable disease in the State of Colorado.

The question remains what the reasons are for this perceived increase in numbers. Herpesviruses are extremely successful in establishing a long-lasting relationship with their hosts. These viruses are also extremely species-specific, and with their limited host-range, one main strategy for them is '*primum non nocere*' or 'first, do no harm'. Maybe this viral objective needs to be re-phrased into: 'do as little harm as possible' to maintain a secure presence without killing the host. There are three viral characteristics which make this virus-host relationship of co-existence a virtue: i) size of EHV-1 limits the distance for airborne transmission; ii) EHV-1 needs an intact envelope for successful host cell infection, which leads to an inverse relationship between time spent outside the body of a horse and infectivity; iii) EHV-1 is a DNA virus with limited ability to mutate. Hence, EHV-1 has to overcome its weaknesses by evading the host's immune system upon entrance; causing only mild to moderate disease in an organ system with open connection to the environment for an easy spread, horizontal transmission leading to further infections of other horses. Finally, there are latent infections in the host, which allows a stage of hibernation or retreat.

An increasing number of EHM-outbreaks over the last decade have been attributed mainly to a single point mutation in the polymerase-coding gene of EHV-1. This mutation is commonly known as the neuropathogenic vs. non-neuropathogenic mutation, or as a 'single nucleotide polymorphism (SNP)'. EHV-1 strains with this mutation have an amino acid change D vs. N in the viral polymerase enzyme. The D-strain of EHV-1, or neuropathogenic strain, is thought to have some replicative advantages leading to a faster spread in the upper respiratory tract, but replicative advantage may also lead to a higher magnitude and longer duration of viremia in blood leukocytes when compared to the N-strain variant. As viremia is critical for the infection of CNS-endothelial cells leading to EHM it is amenable that a more abundant and longer viremia will subsequently lead to more EHM cases during an outbreak. However, as this mutation already existed in EHV-1 strains isolated during the 1970s, it cannot be the sole reason for an increase in EHM outbreak frequency. In retrospective studies of EHM outbreaks about 75% were caused by the D-strain variant, and 25% were caused by the N-strain variant. This clearly shows N-strain variants are not innocent; N-strains are still capable to cause an EHM outbreak, and that waving the 'all-clear' sign when detecting an N-strain in febrile horses is inappropriate.

Each EHM outbreak is unique in itself. The premises set-up is different, the number of horses is different, and certainly the management is different. However, EHM outbreaks also have a common denominator. EHM outbreaks commonly occur during the cooler periods of the year (October through May). There is usually an index case with a history of fever and/or sudden neurological disease, and there is usually a history for this index case or others in close proximity to the index case undergoing strenuous training or competition elsewhere, and returning to the premises after contact with other horses. If the index case presents with neurological disease typical for EHM, the fever may have been missed in this case. Within 3 to 10 days there will often be more horses with fevers and, although less consistent, with limb edema (see a potential time line for EHV-1 infection in figure 2). Common clinical signs of EHM are an asymmetrical ataxia, dysmetria and weakness in combination with a normal mentation. However, one common finding is impaired urination or a dysuria due to a loss of central control to open the urethral sphincter (classic 'upper motor neuron' bladder).

EHM outbreaks differ as the FRACTION of all FEBRILE horses that will develop clinical signs of EHM is highly variable.

What may be other factors that make us believe there is an increase in EHM outbreak frequencies?

Close contact among horses facilitates transmission of EHV-1. This is probably the main reason why we see EHM outbreaks, world-wide, more commonly during the colder periods of the year (October – May for the U.S.A. or the Northern hemisphere). Fall is also the time of intense commingling of horses (round-ups, sales, indoor competitions). The likelihood of close contact under these circumstances is increased, and, in addition, an indoor environment with limited temperature changes may also cause a longer virus survival once it is outside of a horse's respiratory tract.

Further potential explanations include that the number of horses in 'urban environments' has increased over the last 2 decades, and with this urban increase came a change in horse husbandry. It is no longer the family-owned horse, housed on a ranch with a few others and only an occasional visit to a local rodeo or competition. For example nowadays, horses maybe 'boarded' in large-scale facilities where some 200 horses maybe 'Under One Roof'. These two hundred horses are owned by about 200 different owners with differences in opinion on general horse care and management. For performance horses there is a wider selection in competitive styles, the show-circuit has become more intense with competitions that are hosting larger number of horses; competitions are conducted more frequently during a show season with horses going from competition to competition without returning to their home base, and they are spanning more days than in the past. All these factors may contribute to enhanced transmission of EHV-1 from one horse to another.

There is one more argument that helps to explain the perception of higher numbers of EHM outbreaks per year, which is increased 'detection' which is facilitated by a heightened awareness of the disease, more reporting of disease when it occurs and enhanced testing methods. There is the introduction of new communication tools such as blogs, twitter and professional list serves that have lead to enhanced awareness of EHM and other equine diseases.

What are risk factors for EHM?

While infection, respiratory tract replication, fevers and viremia are commonly encountered in a large proportion of horses during an EHV-1 outbreak, the proportion of horses with clinical EHM is a fraction of the total number of cases of EHV-1 infection and is rarely larger than 50% of all infected horses and more likely to be less than 10%.
In fact, it is now known that signalment, in particular breed and age, are significant risk factors in the development of EHM. The middle-aged 'tall horse breeds', the Warmblood, Thoroughbred, Standardbred, Draft horse, are more likely to develop EHM following EHV-1 infection than pony breeds or yearling horses. Furthermore, during 40 years of EHV-1 research, there has not been a single description of a confirmed EHM outbreak on an Arabian stud farm in the scientific literature.

Findings of several field-based EHM outbreak studies have been confirmed by experimental infection studies: yearling horses, intra-nasally infected with a high dose of EHV-1 (D-variant strain), are unlikely to develop EHM, while the same dose and strain of virus will cause EHM in 2/3 of infected aged horses. Host characteristics, thus, have to play an important role in the pathogenesis of EHM!

These findings help to explain why there are significant differences between EHV-1 outbreaks in the percentage of horses that developed EHM. Certainly, during outbreak conditions there will be horses with pre-existing immunity through previous natural infection or vaccination that will be able to avert respiratory tract infection and viremia. Under outbreak circumstances there will be mitigation with the purpose of lowering the rate of exposure and thus infection. However, the composition of exposed horses in a facility will also influence the percentage of horses with EHM. A Thoroughbred training stable on a race track, mainly composed of 2 to 4-year-old animals might have fewer EHM cases than a Standardbred race track facility, where typically middle-aged horses are housed. A boarding facility with great diversity in age groups and breeds of horses likely will have a larger prevalence of EHM cases. Multiple biosecurity risk factors (crowding, visitors, common use areas, multiple contacts, social gathering) that facilitate the spread of EHV-1 will increase the number of horses becoming infected and febrile. The breed and age as well as other host aspects yet to be fully understood will determine the percentage of

EHM-affected animals following a fever.

What are current treatment options for EHV-1 cases?

Anti-viral drugs are advocated during EHM outbreaks. Current recommendations are to treat with anti-virals as quickly as possible, and ideally, as soon as a fever is noticed. Anti-virals should be administered as long as the horse is febrile and for an additional minimum of 5 extra days. Antiviral drugs are costly, thus an owner may decide to only treat the most valuable horses that are at increased risk of developing EHM (see above). Vaccinations of potentially exposed horses in the face of an outbreak with any of the currently available vaccines, is not recommended. However, there are some encouraging results from *in vitro* studies, conducted at Colorado State University, that suggest that the prudent use of non-steroidal anti-inflammatory drugs, specifically flunixin meglumine or firocoxib, may aid in decreasing the rate of CNS-endothelial cell infection with EHV-1 during viremia. Non-steroidal anti-inflammatory used in combination with an anti-viral drug during the febrile phase of an EHV-1 infection may provide a powerful combination of drugs in decreasing the frequency of EHM cases during an outbreak.

What is the veterinary practitioner's role in management of EHM?

There are several differential diagnoses to consider when a neurologic horse is encountered. Horses with EHM can have variable clinical signs with the most common being a wobbly gait that can progress to recumbency within a short time. Differentials include Rabies, West Nile virus, Eastern or Western Equine Encephalitis virus, intoxication such as botulism and liver or kidney failure resulting in encephalopathy. These diseases can only be definitively diagnosed through laboratory testing. Some clinical scenarios are less likely to be consistent with a diagnosis of EHM for example if a single horses is affected AND/OR the neurologic signs consist of multiple cranial nerve deficits and/or an altered mentation (including seizures). This description would be more consistent with a diagnosis of a virus infection causing encephalitis, WNV, EEE/WEE,VEE or Rabies.

It is important to acknowledge that all outbreaks begin with a first case. There are some important clues that may increase the veterinarian's suspicion of EHM. These include recent horse movement or commingling of horses, recent (long distance) transport of the horse or other stressful events occurring in the herd. In addition the occurrence of fever in one or more horses in

the herd is an important clue so asking about this in the history taking is important.

It is important to mention that MOST horses by the time they develop EHM are afebrile. Despite the hope that these clues would allow a veterinarian to sort out the differentials not all EHM cases have these clues associated with them. So it is important to pursue an etiologic diagnosis in all neurologic horses: testing!

PCR-testing of nasal swabs and EDTA-whole blood, in combination, will provide a reliable and fast result (for nasal swab sample collection see figure 1). However, there is always a risk for false-negative test results. If the affected horse's history, signalment and current presentation support a diagnosis of EHM despite a negative PCR-result, re-testing is strongly advised as well as testing of febrile horses in the herd. Also, the horse in question should be treated and isolated from others 'as if' affected by EHM. Always collect serum on an affected horse as testing for alternative diagnoses can be pursued (see also figure 2 for a time line of an infection)!

As there have been multiple reports of outbreaks with a combined presentation of EHV-1 – associated abortions and cases of EHM we strongly advise to follow the same principles of testing of the aborted fetus/ fetal membranes, quarantine of the mare with an abortion, and taking temperatures (twice per day) in in-contact horses.

Because EHV-1 is a highly contagious infectious disease agent early recognition of an EHV-1 outbreak or even occurrence of a single EHM case should trigger implementation of a series of measures to limit further spread of infection on the premises or beyond. Suspect EHM cases should be reported to the State Animal Health Official in Colorado so that they can assist in directing next steps in containment of the disease.

1. Quarantine: the horse operation should be quarantined, and no horse should leave the premises until the risk of transmission of infection has ended. Contact between horses should be avoided, and the importance of fomite transmission for EHV-1 should be recognized.

2. Biosecurity: each horse should be handled with personnel wearing individual protective gowns, footwear covers and disposable gloves; strict adherence to hand sanitation between horses is essential. Disposable head covers for personnel are also recommended. It is important to regard all EHM cases as infectious for at least 14 days after onset of clinical, neurological signs. Testing is necessary to decide on the shedding status of this particular horse.

3. Confirm the diagnosis: nasal swab samples and whole blood in anticoagulant (EDTA) for PCR testing should be collected from all currently febrile or recently febrile horses and from horses with clinical signs consistent with EHM. Complete postmortem examination should be performed on any cases that die. It is important to alert the pathologist of the suspicion of EHM so that the appropriate samples and tests are performed on the tissues collected at necropsy. Serum from all affected horses and 20% of unaffected horses should be collected on day 1 (day of the index case) and 14 days later (convalescent samples from the same horses) stored at -20°C.

4. Rectal temperatures: taken twice daily can help in following the course of the outbreak and in estimating when it is over. Institute treatments as described above.

5. Criteria for releasing the quarantine can be contentious; however, for EHM situations this will be determined by the Office of the Colorado State Veterinarian. Various criteria have been used, including waiting 28 days from the onset of the outbreak or for 14 to 21 days after the last day of a recorded fever in a horse on the premises. Serial testing of nasal swabs by PCR may be of benefit. However, this is an expensive approach and may not offer any advantages over other strategies. Such PCR- testing approach should not be initiated until at least 14 days after the last recorded fever in a horse considered exposed in the outbreak.

What are precautions that can be taken to avoid an EHM outbreak on a farm?

Strategies for prevention of EHM are the same as for preventing any EHV-1 infection. Biosecurity measures are likely to be the most important in reducing risk as no currently licensed vaccine has demonstrated convincing evidence of its ability to prevent or ameliorate an EHM outbreak. Use of vaccines to reduce viral shedding in conventional EHV-1 infections may have some value. Vaccination in the face of an EHM outbreak may be used in unaffected horses (afebrile and on neighboring farms only). Concerns in the view of some clinicians remain that vaccination may facilitate development of EHM, but at present clear evidence for this is lacking in the literature. New additions to a premises, as well as return of resident horses from an event or show, especially during the 'at risk' season, are strongly associated with EHM outbreaks. Therefore, quarantining such horses and monitoring for fever is a valuable strategy for preventing an outbreak.

What are some advances that maybe coming related to EHM?

There is ongoing work related to the pathogenesis of EHM, vaccine development, therapeutics and studies on the survival times of EHV-1 in the environment, some of which is being conducted at CSU. There maybe future options for detection of horses at risk for EHM based on testing of CSF prior to the development of neurologic signs thus allowing more targeted treatment of at risk horses. In addition there was a proposal at the recent U.S. Animal Health Association/American Association of Veterinary Laboratory Diagnosticians that there be a comparison of PCR test performance across laboratories performing the PCR test for EHV-1 so that practitioners and regulatory veterinarians would have a better understanding how the various test methods compare, so stay tuned.

Take home message:

For the time being it is important that veterinary practitioners are vigilant in pursuing an etiologic diagnosis for neurologic equine cases recognizing that outbreaks of EHM all start with a first case. The State Veterinarian's office and equine faculty at CSU (Drs. Landolt and Goehring) are interested in supporting the efforts of veterinarians in the field should there be a need for reporting of suspect EHM cases or consultation about workup or other management of equine neurologic cases respectively.

For those wanting more detailed information on EHM the following are suggested resources

AAEP website has guidelines on infectious diseases including EHM at:

http://www.aaep.org/control_guidelines_nonmember.htm

ACVIM has a consensus statement on EHM at:

http://www.acvim.org/websites/acvim/index.php?p=22

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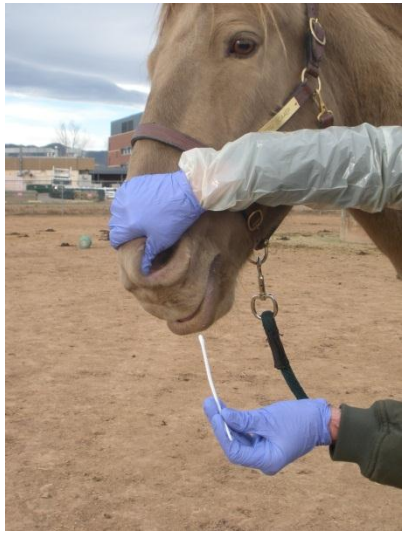

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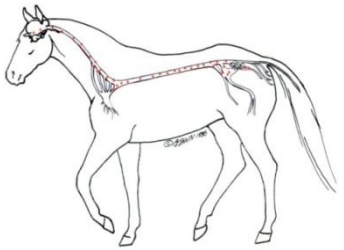
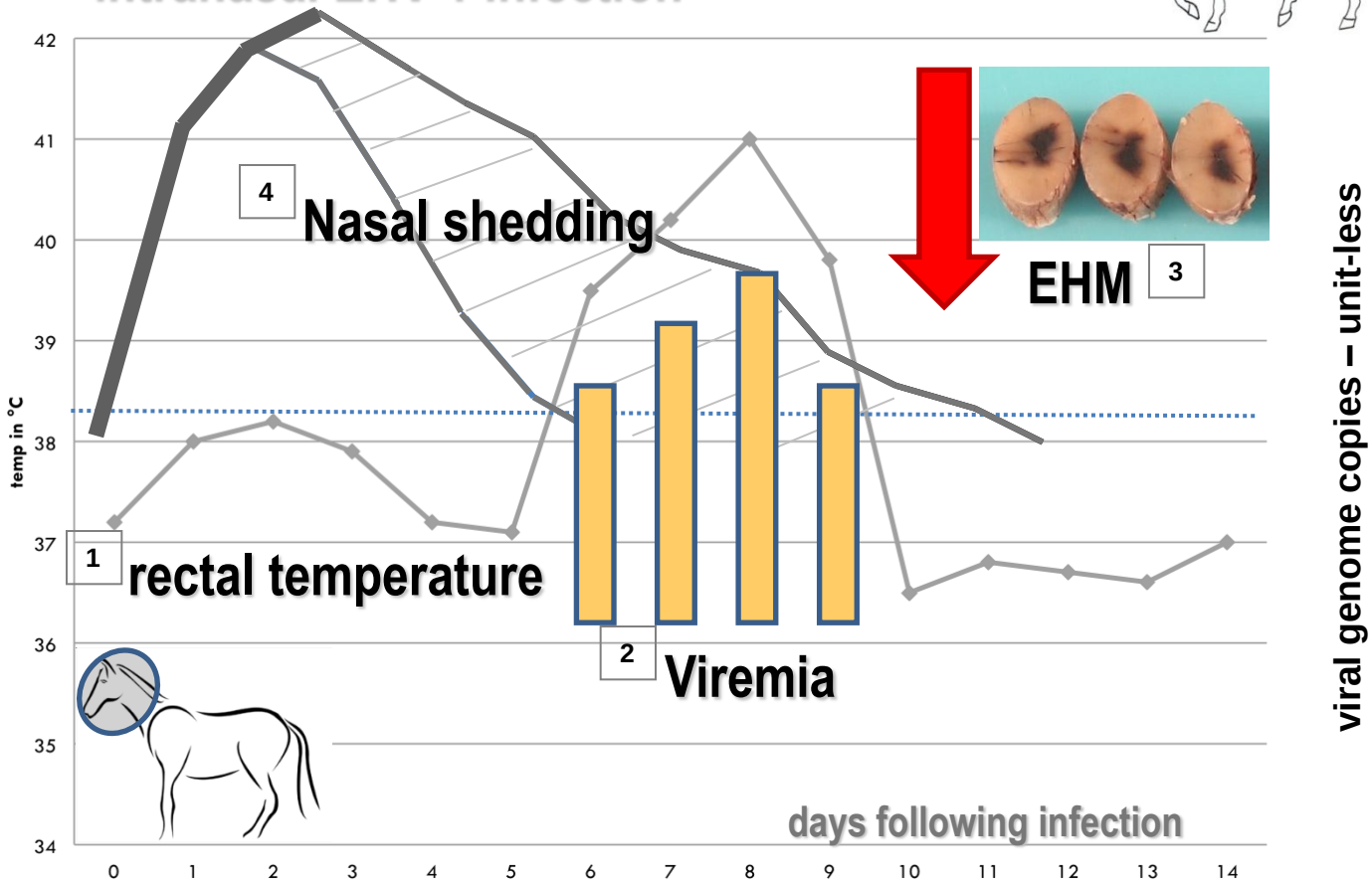
1- Dacron(TM) – or Polyester-tipped swabs; 2- with a bendable, plastic shaft; 3- wear disposable gloves and change between horses; 4- place your thumb against the nasal septum and grasp with your fingers across the nasal plane; 5- quickly insert both swabs in the nasal passage.Go in-and-out for at least 3 – 4x. Make sure there is contact with nasal mucosa; 6- swabs show some signs of celular debris; 7- for PCR analysis swabs should go into an empty transport container, plastic shaft can be broken off to allow the tube top to be secured; 7-label each tube with horse and client ID.



A time line following experimental intranasal EHV-1 infection



This figure illustrates findings of nasal shedding; rectal temperature; viremia, and the (potential) occurrence of EHM following an **experimental** intranasal infection with EHV-1. X-axis: time in days; infection on day=0; Y-axis: rectal temp.in °C; alternative Y-axis: unit-less scale for viral genome quantities; dotted line: fever cut-off (38.3°C-101.3°F). 1- rectal temperature curve is often bi-phasic. Secondary fever is associated with 'cell-associated viremia'; 2- cell-associated viremia, duration 3 – 5 days as determined by PCR; 3- clinical EHM usually follows viremia; 4- nasal shedding is high during the first 3 days and may be associated with a primary fever. Duration of nasal shedding varies significantly between horses, which is represented by the area between the 2 lines of nasal shedding.