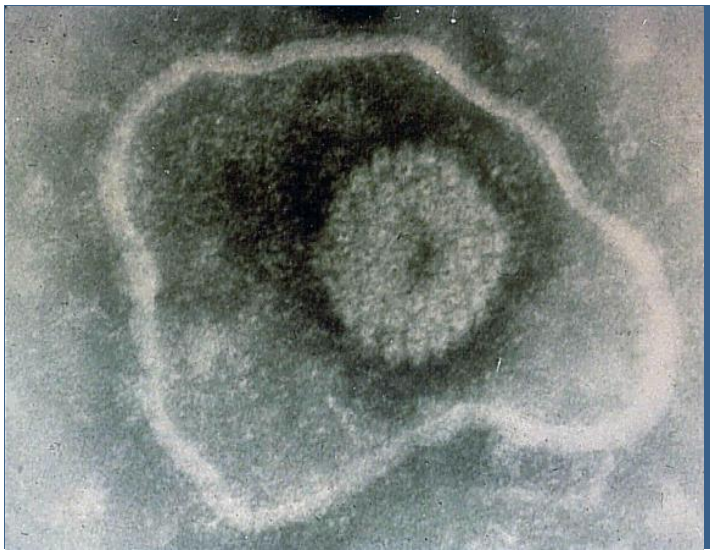
EFTBA Veterinary Newsletter 8



EHV-1/-4 Equine Herpes Myeloence-phalopathy (EHM)

November 2012

- *More detailed informations on all aspects of the neurologic (or paralytic) form of EHV-1 infections, the myeloencephalopathy (EHM).*

Welcome to EFTBA's veterinary newsletter

One of EFTBA's main aims is to inform national associations and indeed breeders themselves of latest developments which affect their business. Health and disease are of course crucial factors for a thoroughbred stud farm. EHM is a disease which spreads rapidly and can occur anywhere and anytime throughout Europe. Therefore by informing and alerting breeders to the specifics of this disease we hope that we can assist in its early identification and help to prevent its spread. Once again I would like to thank Hanspeter for another fascinating veterinary newsletter.

Rhydian Morgan-Jones

Chairman, EFTBA

Editorial

After some basic informations on the nature of herpesviruses in newsletter 7, with this number we want to highlight our great concern about the growing importance of the paralytic form of EHV-1, the equine herpesvirus myeloencephalopathy (EHM). Here, prevention is of utmost importance as the spread of the infection occurs easily and we still don't have efficient means for therapy.

Dr Hanspeter Meier

EFTBA Delegate (Switzerland) and veterinary advisor & Newsletter editor

Summary

Among the different diseases caused by EHV-1, the equine herpesvirus myeloencephalopathy (EHM) is a life-threatening form which increased worldwide in regard to the number of severe and costly outbreaks. Its therapy is either only symptomatic or must, if antiviral drugs are used, be initiated very early in the course of the disease. The cause is attributed mainly to a nucleotide polymorphism in the DNA polymerase gene, where Adenine is replaced by Guanine. However, it would be overly simplistic to attribute the pathogenesis to this fact only.

"Many thanks to Mrs. Eva-Maria Bucher-Haefner, Moyglare Stud Farm, for her valued sponsorship of this newsletter."

Profound Beauty (Danehill) owned and bred by Moyglare Stud.

Morevoer, these mechanisms are not fully understood yet and both host and environmental factors are supposed to play important roles also. Prevention is of utmost importance, above all a strict vaccination policy and rigorous control measures (e.g. biosecurity measures as quarantine and isolation). Better surveillance and monitoring in non-breeding horses and non-Thoroughbred populations should be encouraged.

Introduction

According to Kydd and co-workers (2010), equine herpesvirus myeloencephalopathy (EHM) has been described for many years, but recently there has been an apparent increase in the number of reported outbreaks in the USA, from one in 2001 to eleven in 2006 (USDA report 2008; Table 1, p. 64). The best known of these was a disastrous EHM outbreak in Ohio in 2003, affecting over 100 horses (Henninger et al. 2007). In the United States, the unexpected increase in incidence over the past decade of EHM, has led to declare the neurologic (or paralytic) form of EHV-1 infection an emerging disease there in 2007. Beside this, the economic importance of EHV-1 disease is considered as "one of the costliest equine diseases worldwide" (Allen et al. 2008, Perkins et al. 2009, Vissani et al. 2009, Fritsche and Borchers 2010, Pronost et al. 2010, Smith et al. 2010, West 2011, Tsujimura et al. 2012).
According to reports from the US, the problem is ongoing, as two horses died at Hawthorne racecourse in the second half of October 2012.
Here in Europe, the situation doesn't seem to be so dramatic yet, but we still read some notifications now and then, e.g. two cases of the neurologic form in riding horses in France last month.
It therefore would be irresponsible if one wouldn't also be most attentive and careful in observing the development and enforce means for prevention. For this we want to get familiar with our possibilities as breeders.

The EHM in Europe

Almost 50 years ago, the "isolation and identification of equine rhinopneumonitis virus from cases of abortion and paralysis" was reported from Saxegaard (1966) in Norway, for the first time in Europe. Shortly afterwards (1969/70), paresis and paralysis in mares were also reported in two German studfarms, and another two reports were made there in 1980 and 1981 (Merkt and Petzold).
According to Timoney (2008), in 1972, the Central Veterinary Research Laboratory of the Irish Department of Agriculture followed a series on major outbreaks of EHM that affected the local Thoroughbred breeding industry that year.
Greenwood and Simpson (1980) reported a paralytic syndrome affecting stallions, mares and foals on a Thoroughbred studfarm in England, where in the year before within few weeks 2 of 4 stallions, 5 of 39 foals and 35 of 39 mares were affected. Nine mares died or had to be destroyed.
Severe cases were also reported from Austria, where the world-famous Lipizzaner-horses were afflicted (Bürki et al. 1984). Further on, an outbreak of paresis occurred among 67 Haflingers in India, which had been imported by the Remount and Veterinary Corps in 1982 from Austria (Tewari and Prasad 1989).
Switzerland reported ten assumed and confirmed cases from 1991 till 2010, but possibly both here and in other countries, the time lag in comparison to reports from abroad may be due to the lack of disease-awareness and to the markedly smaller equine population.
Very interesting and useful studies were made in Belgium (Van der Meulen et al. 2003) and especially in The Netherlands (Goehring et al. 2006). There, EHM has been considered the most prevalent infectious cause of neurologic disease among an estimated population of 400'000 horses and ponies since the 1980s. This has to be considered a remarkable observation, as it is the result of incomparably sophisticated investigations.
In Eastern Europe, two outbreaks were reported from Croatia in 2009, after the import of American Quarter Horses and the removal of an Arabian stallion to another farm (Barbic et al. 2012).

The few references as above do not claim completeness at all, especially as small-scale outbreaks often will not be noticed by the public, and even may not be reported to avoid stigmatization of the premises (Goehring et al. 2006). We only want to show that EHM may not only occur anywhere and anytime in Europe and that the virus is latent in many other populations than Thoroughbreds. The risk of infection is great, especially as surveillance, monitoring, control and prevention outside the TB-industry still doesn't satisfy.

Symptoms

The course of the equine herpesvirus myeloencephalopathy is often quite dramatic, as normally there are no or only minor premonitory clinical signs of respiratory disease, and **pyrexia** is likely to be the only warning symptom. Clinical signs usually appear suddenly and reach their peak intensity within 2 to 3 days of onset. Neurologic dysfunction ranges from temporary ataxia and paresis to complete paralysis. The hindlimbs are usually the most severely affected (fig. 1), although quadriplegia has also been observed. Bladder dysfunction, characterized by atony with incontinence or urinary retention, and cutaneous perineal and limb sensory deficits result from sacral nerve involvement. Some affected horses develop a head tilt. The prognosis for non-recumbent horses is favorable, but is poor for recumbent animals, which frequently develop fatal complications, e.g. extensive myopathy, pneumonia, intussusception, bladder rupture and require euthanasia. Neurologic clinical signs appear during or toward the end of the viraemic phase of infection (similar to pathogenesis of abortion). The presentation and severity of clinical signs are highly variable and depend on the extent and location of the neurologic lesions (Slater 2007).

In a very detailed and well done study of Goehring et al. (2006), symptoms of proximal airway infection were subtle, and included mild (serous) nasal discharge and lymphadenopathy. Ventral trunk or limb edema was encountered regularly, but always less frequently than fever. Regarding visible edema, in the Dutch investigation, members of the draught breeds and older horses were at increased risk (Goehring et al. 2006).

Older horses, females and animals with fever were more at risk to develop signs of severe neurologic dysfunction in the investigation of Goehring et al. (2006). Standardbreds and draught horses were more likely to develop fever, compared with archetypical ponies. Neither sex nor age contributed to the risk of developing fever.

The number of days of fever and its magnitude did not seem to correspond with the occurrence or severity of clinical signs of EHM (Goehring et al. 2006). This peculiar fact could also be observed in an outbreak in Switzerland (Meier 2010).

In regard to the age of the patients, Allen (2008) clearly showed that age was extremely important in influencing expression of neurological disease. In his study, older horses (>20 years) were more predisposed to the development of high titre viraemias and neurological disease when experimentally exposed to a highly neuropathogenic strain of EHV-1. In contrast, when young to middle aged horses (<15 years) were infected under identical conditions, they were 8 times less likely to develop neurological disease and their viraemia titres were on average 100 times lower than those detected in older horses. It is well possible, that the effect of age may be closely associated with the immunological status of the animals.

Figure 1: The neurologic dysfunction usually affects the hindlimbs most severely but can also lead to quadriplegia and recumbency with poor prognosis (www.ca.uky.edu/gluck/BiblioEHV1/asp)

Diagnosis

Considering the sudden appearance with almost no premonitory symptoms (just the same as with abortions), usually the first case(s) of EHM just occur ('index animal(s)'). Other reasons for neurological problems do exist, of course, but a herpesvirus-infection must always be kept in mind and the body temperature of the befallen and other horses must be checked. This cost-effective procedure is of great importance, but – probably because of its simplicity – is far too often under-estimated and neglected, and its documentation as well (fig.2).

The next step is trying to isolate and/or detect the virus by polymerase chain reaction (PCR) assay. For this procedures serves a nasal/pharyngeal swab (fig. 3) or a blood collection. A PCR-investigation normally delivers a fast result, which is most welcome with a suspicion of EHM.

Finally, one can also collect blood samples 2 to 3 weeks apart for measuring the levels of antibodies specific to EHV-1. This investigation has to be done at the same time and therefore, one gets the results only late; they are more of scientific than clinical meaning.

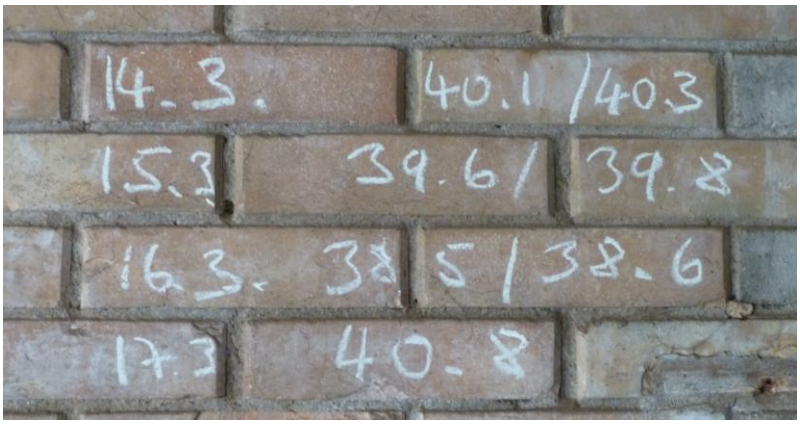


Figure 2: Even the simplest mean of documentation of pyrexia (often the only premonitory clinical sign) is very valuable

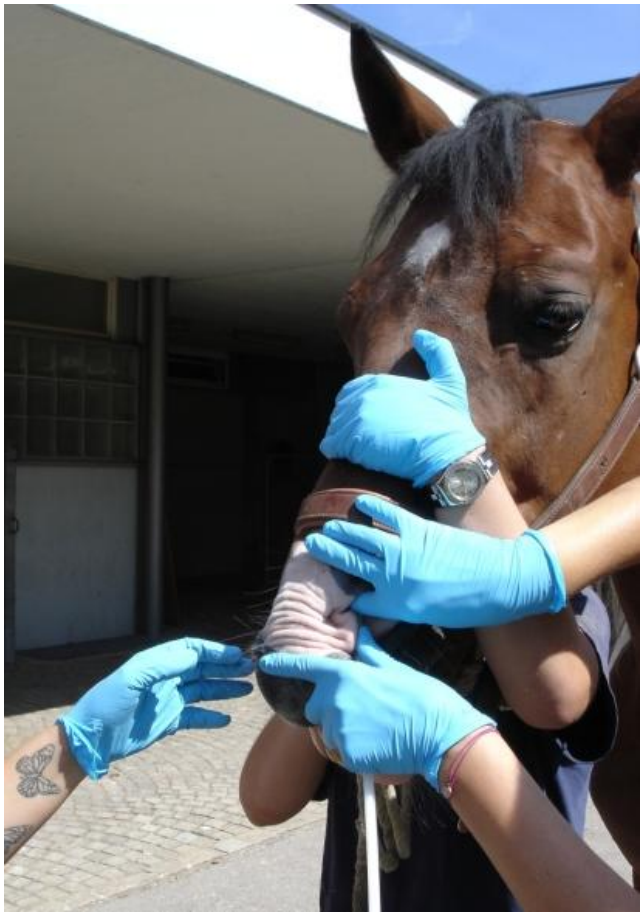


Figure 3: The collection of a nasal/pharyngeal swab

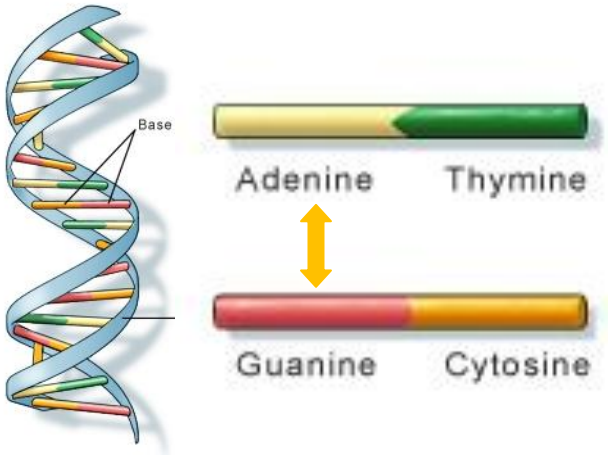
Etiology and pathogenesis

According to Slater (2007) and West (2011), the interval between infection and subsequent onset of neurologic disease (incubation period) is usually between 6 and 10 days but may be as short as 1 and as long as 14 days.
All current data suggest that EHV-1 myeloencephalopathy develops as the consequence of endothelial cell infection in the brain and spinal cord, due to cell-associated viraemia, arising either from reactivation of latent infection as a result of stress (e.g. transport, athletic exertion, management change) or acquired respiratory tract infection. Neuronal infection is rare (representing a significant difference from other encephalitic herpesviruses), with neurologic signs developing as the result of vasculitis, thrombosis, and secondary ischemic degeneration of the neuropil (neuropil, sometimes referred to as "neuropile," is a broad term defined as any area in the nervous system composed of mostly unmyelinated axons, dendrites and glial cell processes that forms a synaptically dense region containing a relatively low number of cell bodies; the most prevalent anatomical region of neuropil is the brain) (Kydd and Smith 2006).
During an observational period of 4 years, Goehring and colleagues (2006) evaluated 9 outbreaks of EHM in the Netherlands (195 horses). They also think that clinical signs of neurologic dysfunction may be the result of an endothelial cell infection of the central nervous system, with accompanying thromboembolic disease and ischemia, particularly involving the spinal cord. Microthrombosis and vasculitis may be consequences of the immune system attempting to clear infection by humoral and cellular mechanisms.
Infected horses usually will shed virus into the environment for seven days or less, but in some cases they can shed for two weeks or longer (West 2011).

Genetics / Genomics

Genetics, one of the favourite subjects of the TB-breeder, play also a big role for the understanding of the different actions of herpesviruses. Principally, EHV-1 can be divided into several major genetic subgroups, even with a geographic restriction between Europe and North America. In regard to EHM, our special interest in this newsletter, the DNA-sequence of EHV-1 strains isolated from horses showing either neurological or non-neurological disease, were compared by Nugent and co-workers (2006). These studies suggested that **EHM** is associated with **a single nucleotide polymorphism** at position 2254 in the EHV-1 DNA polymerase gene. It is **a point mutation** of the two nucleotides **Adenine** and **Guanine**. EHV-1 virus isolated from horses, that did not show neurological disease, possessed the nucleotide Adenine (A2254) and coded for asparagines (N752). In contrast, in the paralytic, mutated strain, the nucleotide Guanine (G2254) was present which translated to aspartic acid (D) at the position 752 (D752) in the DNA polymerase protein (Nugent et al. 2006) (fig. 4).
Based on these findings, EHV-1 strains possessing Guanine (G2254) at this site are considered to have neuropathogenic potential, whereas those strains with Adenine (A2254) are thought to be non-neuropathogenic and usually, but not invariably, associated with abortion and respiratory disease in horses. Follow-up work with different isolates and subsequent nucleotide substitution experiments (using molecular clones) did not only confirm this discovery but could even demonstrate the greater pathogenicity of the D752 biovariant (Goodman et al. 2007; Allen 2008, van de Walle et al. 2009; Vissani et al. 2009, Ma et al. 2010, Pronost et al. 2010a).
Furthermore, Perkins et al. (2009) performed a statistical analysis from 176 EHV-1 isolates and demonstrated that the odds of neurological disease being associated with the G2254 genotype were 162 times greater than those with the A2254 genotype.
Finally, the recently observed increased incidence of EHM correlates with the higher prevalence of viruses with a G2254 genotype currently being isolated in diagnostic laboratories in Europe and the USA (Perkins et al. 2009; Pronost et al. 2010b; Smith et al. 2010).

We breeders all know well that DNA-sequencing also does allow investigations into the past, e.g. into historic Thoroughbred horses as for instance Bend Or (Campana et al. 2010, Morris 2011). A comparable analysis was done with a large panel of archived EHV-1 isolates collected from sporadic cases of equine abortion between 1951 and 2006 in Kentucky, using a real-time Taq-Man allelic discrimination PCR. This investigation revealed that viruses with the G2254 neuropathogenic genotype existed at least as far back as the 1950s – well in accordance with the clinical observations as mentioned above. Furthermore, such isolates increased in prevalence from 3.3% in the 1960s to 14.4% in the 1990s, with indications of an even higher incidence from 2000 onwards (Smith et al. 2010).



U.S. National Library of Medicine

Fig. 4 This tiny part of the DNA serves to explain, that in the mutated, neurologic EHV-1 strain just the base Adenine (A) has been replaced by the base Guanine (G). Adenine codes for asparagines and Guanine translates to aspartic acid (please refer to newsletter Nr. 5, to the basics of genetics/genomics in the horse)

The association between this polymerase mutation and neuropathogenicity asks for an explanation, of course. One biological reason is that strains of virus with the neuropathogenic DNA polymerase mutation may replicate more efficiently in leucocytes and endothelial cells or reactivate more readily from latency, resulting in an increased risk of viraemia and subsequent neurologic disease (Kydd and Smith 2006, Allen and Breathnach 2006, Allen 2008). Testing this hypothesis in weaned foals by Kydd and Smith (2006), the magnitude of EHV-1 viraemia from an isolate associated with neurologic disease was 5-fold greater than an abortigenic strain of EHV-1. This implies that the load of virus transmitted to the central nervous system by the cell-associated viraemia is substantially higher in the strains that cause neurologic disease than those that result in abortion (Kydd and Smith 2006).
However, the 'non-neurological' virus biovariant (A2254/N752) has also been isolated from a substantial number of horses with neurological disease, and Pronost et al. (2010b) also identified a number of G2254 genotype isolates from numerous horses with no evidence of neurological involvement. This suggests clearly that this nucleotide substitution is not the only determinant of neurological disease and additional factors contribute to the onset of EHM (Goehring et al. 2009, Kydd et al. 2010, Pronost et al. 2010b).
According to Pronost et al. (2010b), even with such a tiny being as a virus, possibly there exist also epigenetic factors, which make things even much more complicated. So, it is obvious that there are still unknown factors to the pathogenesis of EHM and at present we must be satisfied with the results of these recent publications. We must confess that we don't understand the genomics of herpesviruses well enough yet. – But I guess there is nevertheless still hope that we will ever understand the genomics of a more complex organism like a racehorse.

Therapy

According to Goehrig et al. (2006) and Slater (2007), treatment of horses with neurologic disease is largely empiric because little experimental or clinical evidence exists to support many of the drugs used. Based on pathologic observations that the nervous system lesion is a thrombo-ischemic injury secondary to virus infection of endothelial cells, many clinicians treat affected horses with non-steroidal anti-inflammatory and antiviral drugs. Supposedly, aggressive therapy with acetylalicylic acid or cortisosteroids at an early stage of an outbreak may possibly influence disease outcome.

Already in 1992, Gerweck and co-workers treated a few horses with the antiviral drug Zovirax® (acyclovir ACV). However, they were successful in very early cases only and mentioned also the high costs

of this treatment (at that time about 5'000 DM). In their opinion the therapy has to be initiated at the time of clinical suspicion already.
Thereafter, ACV has been used in different places of outbreaks of EHV-1 myeloencephalopathy, but although ACV works well enough against human herpesviruses, it is not equally active against the different equine herpesviruses and less effective against EHV-1. Slater (2007) says that the current enthusiasm for its use should be tempered by the paucity of in vitro or in vivo experimental evidence of its efficacy. In his opinion, the absence of data from controlled clinical studies in affected horses raise questions about the rationale behind its use. Moreover, the estimated costs are still approximately € 50 per day (Slater 2007). Further explanations for the limited value of antiviral therapy with ACV were already made by Kydd and Smith in 2006: In clinical cases, the viral replication, which we want to prevent with ACV, generally already has ceased by the time that clinical neurologic signs have developed. Beside this, in human medicine, ACV-resistant isolates of herpes simplex virus have been characterized (Sauerbrei et al. 2010).
Nevertheless, the balance of clinical opinion is that ACV treatment appears to be worthwile. One just has to bear in mind, that ACV interferes with virus replication by preventing viral DNA synthesis – and therefore must be used in a very early stage (as already recommended by Gerwick et al. 20 years ago).

Another method for treating EHV-1 infections was examined by Brosnahan et al. (2009). They tried the novel technology of RNA interference which they think to show promise for use in outbreak situations. According to the mode of action, these molecules are called siRNA and "si" stands for "small" or "short interfering" and also "silencing" RNA (the last is probably the best expression). But whatever one calls them, they have only 19 to 23 pairs of bases, but nevertheless are able to interfere with the gene-expression of cells and viruses and silence them so. This type of RNAs has only been discovered a few years ago in plants (Baulcombe 1999) and their effect in mammalian cells two years later (Tuschl 2001). The group of Brosnahan (2009) examined the effect of siRNA on nasal shedding, viraemia, and clinical signs in experimentally infected horses. 12 h before and 12 h after intranasal infection with neuropathic EHV-1, one group of horses received siRNA-targeting EHV-1 genes, and the control group got a different siRNA (a firefly luciferase) from which no effect could be expected. There was no significant difference in viral shedding and viraemia, and in body temperatures significant differences occurred only on days 6 and 7. However, neurologic signs developed in 3 of 4 control horses and only in 2 of 10 horses receiving EHV-1–targeted siRNA. Neurologic scores were also significantly lower in this group of horses from day 9 onward. In conclusion, Brosnahan et al. (2009) stated that metaphylactic, intranasal administration of equine herpes virus-targeted siRNA decreased severity of fever and neurological signs relative to controls in experimentally infected animals. But nevertheless, they also consider that further research is necessary to define the relative contributions of host and virus factors, as the etiology of EHV-1 neurological disease is complex (Brosnahan et al. 2009).

For further and detailed technical informations on the action of silencing RNA – please refer to siRNA-YouTube.

Epidemiology

Slater (2007) describes the neurologic form a sporadic and uncommon but potentially devastating manifestation of EHV-1 infection. Large outbreaks can occur, affecting 30% to 40% of horses on the premises. EHV-**4** paralytic disease is rare, but isolated cases have also been identified.
Transmission is assumed to be through the respiratory route, although the source of infection may also be endogenous reactivating virus.
In regard to the epidemiology, with 9 confirmed outbreaks and compared with other diseases, EHM was in the Netherlands the most frequently encountered neurologic disease in a group of horses during the observational period. Most interestingly, it also was the most consistently reappearing disease each year and there was strong seasonal clustering, with all outbreaks occurring between mid-November and mid-May (Goehring et al. 2006).
These findings stress the influence of 'season' and suggest that ambient temperature, humidity, or other environmental factors are important for viral spread. Crowding of horses and possibly increased survival of the virus in the environment during winter and spring may be the reason for clustering of EHM outbreaks during these months.

Japanese researchers were unable to document EHV-1-specific seroconversion in their racehorses during summer months either, despite year-round EHV-**4** seroprevalence or seroconversion (Yasunaga et al. 1998). - Season really seems to be another environmental factor which may be a piece of the pathogenetic puzzle.

The typical presentation of EHM in The Netherlands was:
- An index animal
- fever in larger numbers of horses than in those with signs of neurologic dysfunction
- signs of mixed neurologic myelopathy
- specific season (late fall, winter, spring, with few exceptions)
- affected horses more likely associated with specific risk factors (breed, age, sex).

The significance of fever, as already stressed as a very important symptom, was highlighted once more with a multivariate analysis by Goehring et al. (2006). Fever was the only parameter that remained in the formula, suggesting that fever is the most important feature related to the odds of developing signs of severe neurologic dysfunction.

In the study of Goering et al. (2006), Standardbreds, members of hispanic and draught breeds were more at risk to manifest signs of neurologic dysfunction. In contrast, these researchers never observed EHM in the archetypical pony breeds, Fjord, Icelandic Horses and Haflingers. However, the latter finding is not in accordance with the investigation of Tewari and Prasad (1989); this may be due the low figure of these ideal pack-animals in the Netherlands and once again emphasizes the still poorly understood pathogenesis of EHV-1 infections and contributing environmental factors.

Even though there was no age predisposition to develop general neurologic disease, it was not detected in Dutch horses < 3 years of age. This is not in accordance with the findings of Greenwood and Simpson (1980), but doesn't contradict it either. Most probably, it just points to the fact that the pathognesis must be pretty complex, that many other (e.g. environmental) factors also seem to play a great role. Just remember, herpesviruses are very special and most interesting beings in any respect.

In the last newsletter, we explained the difficulties of immunising efficiently against herpesviruses generally and, naturally, these unfavourable facts render the prevention of paralytic cases also very demanding. However, the investigation of this aspect was not possible for the group of Goehring (2006), as only five horses (out of 195) had received a single vaccination at a previous time in their lives and only another five had a current vaccination status (!) None of the few vaccinated horses were observed to develop neurologic disease, but these small numbers of vaccinated animals did not allow further analysis, of course.

Control

Considering the duration of shedding of the virus (possibly longer than two weeks), the AAEP recommends a 28-day quarantine period for ill/exposed animals. In some states, officials might allow a shortened quarantine period of 14 days if animals "test clean" via real-time PCR. However, in the end it might be cheaper to simply continue to board horses at the quarantine location until 21 days have passed since the latest clinical case was identified or became asymptomatic (West 2011).

The role of host immunity in influencing the severity of the neurologic signs is incompletely defined, although experimental work carried out by Neil Edington and colleagues at the Royal Veterinary College in 1980s revealed the appearance of circulating immune complexes in the bloodstream of horses infected with EHV-1 shortly before the development of neurologic signs (Kydd and Smith 2006).

As mentioned in newsletter 7, commercial vaccines and vigorous implementation of hygiene measures reduced the incidence of EHV-1 abortion in different places. In contrast to this positive fact, no vaccine is licensed currently to prevent EHM. This is due to different unfavorable factors, e.g. to the unavailability of a suitable animal model that consistently reproduces the desired clinical disease and to the slightly different genetic make-up of the EHM-strain (Kydd et al. 2010).

Nevertheless, the two principal aims to reduce the spread of virus between horses and its dissemination within the infected horse are the same as with EHV-1 abortion. To achieve these aims, the rapid implementation of effective biosecurity measures in the event of an outbreak and a widespread vaccination policy are crucial factors (Kydd and Smith 2006, Meier 2010).

In general, the Thoroughbred breeding industry has a robust voluntary vaccination policy that should be extended more widely to Thoroughbreds in training. As highlighted in the papers of Goehring et al. (2006) and Barbic et al. (2012), given the increased risk of certain breeds developing neurologic disease, the policy must also extend to other sport horse and breed societies.
In the latter respect, especially the investigation of Hebia et al. (2007) finds further interest, as there exists a potential risk of transmission of EHV-1 even by equine embryo transfer.

Although no vaccine is licensed to prevent neurologic disease, current products that contain killed virus are effective at stimulating high titers of virus-neutralizing antibody that bind infectious virus particles, reducing the amount and duration of virus shed from the nasopharynx. The knock-on effect is to reduce the viral load circulating in the equine population. A widespread vaccination policy, even among nonbreeding horses, is therefore to be actively encouraged (Kydd and Smith 2006, Meier 2010).

Readers are cautioned to seek advice of a qualified veterinarian before proceeding with any diagnosis, treatment ot therapy.

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