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EMERGENCE OF EQUINE HERPES VIRUS 1 MYELOENCEPHALOPATHY: A BRIEF REVIEW

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ABSTRACT

Equine herpesvirus 1 (EHV1) is an economically important viral pathogen of equines and causes respiratory disease, neonatal foal mortality, late-term abortion and sporadic encephalomyelitis *aka* equine herpes myeloencephalopathy (EHM) in affected horses. The nervous form of EHV1 (EHM) has been recognized as early as 1950s in horse population; however, many aspects of this disease remained poorly understood. In recent years, there has been much progress in our understanding of genetics, epidemiology and pathogenesis of EHM through close monitoring of field outbreaks in different parts of the world. Various host, agent and environmental factors have been found to a play a role in the development of EHM, the most significant being the identification of a single nucleotide polymorphism in DNA polymerase gene (A_{2254} to G_{2254}), which imparts neuropathogenic potential to the virus. EHM affects horses of all ages, including un-weaned foals and produces clinical symptoms that are indistinguishable from other viral encephalitis/ central nervous system (CNS) disorders. EHM treatment includes supportive therapy, and reducing inflammation of CNS. Diagnosis of affected horses and monitoring of in-contact animals is the best measures to prevent EHM outbreaks. This review in brief discusses about progress made in epidemiology, pathogenesis, treatment, prevention and control of EHM.

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1 Introduction

Equine herpesvirus 1 (EHV1) is a highly contagious respiratory pathogen associated with a variety of disease conditions in horses. It is estimated that 80 to 90% of horses have been exposed to EHV1 infections by two years of age (Allen, 2008). EHV1 infection causes upper respiratory tract infection in young horses, abortion in pregnant mares, neonatal foal mortality and neurological disorders. Abortion is the most economically crippling outcome of EHV1 infection with 95% of EHV1 associated abortions occurring in the last four months of pregnancy. Respiratory disease associated with EHV1 is most commonly seen in young animals at the time of weaning (Allen, 2008). Neurological disease associated with EHV1 is called equine herpesvirus myeloencephalopathy (EHM). Although clinical form of EHM is less frequently observed, it can cause serious economic losses in breeding horses and has very negative impact on equine industry (Friday et al., 2000; van Mannen et al., 2001; Henninger et al., 2007; Pronost et al., 2010).

During past decade, incidence of abortion and rhinopneumonitis due to EHV1 has been declining, possibly due to widespread vaccination practices. At the same time, there has been rise in incidence of EHM in many parts of world *viz.*, Europe, North America, South America, Africa and Oceania (Perkins et al., 2009; Vissani et al., 2009; Pronost et al., 2010; Smith et al., 2010; Fritsche & Borchers 2011; Tsujimura et al., 2011, Cuxson et al., 2014; Negussie et al., 2015; McFadden et al., 2016). The neuropathogenic strains of EHV1 (causing EHM) have also been reported from Asian countries such as Japan (Tsujimura et al., 2011) and India (Unpublished data). This article discusses the aetiology, pathogenesis, epidemiology of EHM and gives an overview of prevention, control and treatment of EHM.

2 Etiology

EHV1 is an enveloped, double-stranded DNA virus belonging to the genus *Varicellovirus* of the subfamily *Alphaherpesvirinae* within family *Herpesviridae* (Davison et al., 2009). As many as nine equine herpesviruses (EHV 1-9) species have been known to infect equines. Among these only five (*viz.*, EHV1, 2, 3, 4 and 5) have the ability to produce diseases in horses. EHV3 is responsible for equine coital exanthema while EHV1 and 4 are the economically important viruses affecting the horses globally (Davison et al., 2009) with EHV1 capable of even causing abortion and neurological disorders as compared to EHV4 (Patel & Heldens, 2005; Lunn et al., 2009). EHV2 and 5 do not cause any specific diseases but remain associated with upper respiratory tract diseases, immunosupression, general malaise and poor performance (Thein 1978; Belak et al., 1980).

EHV1 genome is 150 kbp linear double-stranded DNA composed of a unique long region and unique short region flanked by inverted repeat regions, the terminal repeat and the internal repeat regions. The EHV-1 genome encodes for 76 open reading frames (ORFs). EHV1 isolates have special virulence markers, which are thought to induce EHM.

3 Equine herpesvirus myeloencephalopathy

EHM was present in equine population as early as 1950s, however, its importance came to limelight in the last decade after large outbreaks of EHM occurred in Europe and America (Perkins et al., 2009; Vissani et al., 2009; Pronost et al., 2010; Smith et al., 2010; Fritsche & Borchers 2011; Pusterla et al., 2012; Damiani et al., 2014; Stasiak et al., 2015). Neurological disease can affect horses of all ages, including un-weaned foals, and often requires euthanasia of affected animal Horses exhibiting neurologic diseases can shed the virus in their nasal secretions and transmit the disease to in-contact animals (Henninger et al., 2007).)

The ORF30 spanning the nucleotide region 51522-55184 (3662 nt) in EHV1 genome encodes for a protein referred to as *Pol*, the putative DNA polymerase catalytic subunit which possesses DNA synthesis activity. This gene is highly conserved throughout its length. Recently, a single nucleotide polymorphism (SNP) of guanine (G) for adenine (A) at 2254 nucleotide position of the ORF30 region resulting in an amino acid variation, from asparagine to aspartic acid (N/D752) have been proven to be associated with the neuropathogenic potential of the EHV1 strain (Nugent et al., 2006). This DNA polymerase enzyme of EHV1 has two sets of identical protein subunits each of which contains two catalytic pockets (Liu et al., 2006), serving as site for polymerase activity and the site for 3'- 5' exonuclease activity. In EHV1, neuropathogenic strains, the point mutation results in a switch from no charge to a negative charge and induces a conformational change within the viral polymerase structure and thereby increases the replicative capacity of the virus and produce significantly higher viral loads (Nugent et al., 2006; Liu et al., 2006).

4 Prevalence of neuropathogenicty

Increased numbers of EHM cases have been reported from various parts of the world during the last decade with majority of them from Europe and North America. Europian countries *viz.*, France (Pronost et al., 2010; van Galen et al., 2015), Germany (Fritsche & Borchers, 2011; Damiani et al., 2014), Belgium (van der Meulen et al., 2003; Gryspeerdt et al., 2011), Poland (Stasiak et al., 2015), Netherlands (Goehring et al., 2006) and Croatia (Barbic et al., 2012); North American countries *viz.*, Canada (Burgess et al., 2012) and U.S.A (Nugent et al., 2006; Henninger et al., 2007; Perkins et al., 2009; Smith et al., 2010; Pusterla et al., 2012); South American countries *viz.*, Brazil (Mori et al., 2011) and Argentina (Vissani et al., 2009); Asian countries *viz.*, Turkey (Yilmaz et al., 2012); Japan (Tsujimura et al., 2011) and India (Unpublished data); Islands *viz* ., Australia (Cuxson et al., 2014) and Newzealand (McFadden et al., 2016); African countries *viz.*, Ethiopia (Negussie et al., 2015) experienced

outbreaks of EHV1 infection by neuropathogenic strains of EHV1. The incidence of neuropathogenic genotype from cases of neurological illness reported from different countries varies between 20% and 86% (Perkins et al., 2009; Vissani et al., 2009; Pronost et al., 2010; Fritsche & Borchers, 2011; Cuxson et al., 2014). The prevalence of neuropathogenic strains in abortion outbreaks varies between 1.5% and 25.8%. The percentage prevalence was highest (25.8%) in France (Pronost et al., 2010) followed by 19.4% in U.S.A (Smith et al., 2010), 10.6% in Germany (Fritsche & Borchers, 2011), 7% in Argentina (Vissani et al., 2009), 3.1% in Poland (Stasiak et al., 2015), 2.7% in Japan (Tsujimura et al., 2011) and 1.5% in Australia (Cuxson et al., 2014).

5 Pathogenesis of EHM

Upon entry into the animal body, virus multiplies in the epithelial cells of upper respiratory tract. Following initial replication, the virus spreads to the cells of lamina propria and underlying tissues within 12-24 h, after crossing the basement membrane. By 1-2 days post-infection (dpi), the virus reaches in the local lymph nodes draining the respiratory tract where further replication and infection of leukocytes occurs. Leukocytes harbouring the virus are released to the blood stream (Leukocyte-associated viremia) between 4-10 dpi which enables the virus to reach internal organs including CNS (Kydd et al., 1994; Gryspeerdt et al., 2011). Secondary replication occurs in endothelial cells of CNS-associated arterioles (in particular the vessels of the spinal cord), which may result in nervous system disorders 9-13 dpi. As a consequence, vasculitis, thrombosis, perivascular cuffing of lymphocytes at sites of endothelial infection occurs, probably caused by direct interaction of the host's immune system and infectious agent (Edington et al., 1986). This vascular damage leads to ischaemia and re-perfusion injury of the CNS. Neuropathogenic strains are capable of exhibiting longer and higher level viremia. This high level viremia, interfere the blood flow to CNS and resulting in development of neurological diseases (Fritsche & Borchers, 2011). The exact mechanism by which leukocyte-associated viremia leads to myeloencephalopathy is not known.

6 Clinical Signs of EHM

Onset of clinical signs of EHM usually occur 6-10 dpi following the onset of viremia. Clinical signs depend on number and size of affected sites, as well as relevance and location of affected nervous tissue (caudal spinal cord is most affected). Clinical signs usually include fever, ataxia, paresis/paralysis of hind limbs, bladder dysfunction, urinary incontinence and sensory deficit in the perineal area. In addition ventral oedema, scrotal or preputial oedema in male horses, and limb oedema are also noticed. In severe cases of EHM, paralysis may advance to tetraplegia and death of animal is observed (van Mannen, 2002; Pusterla & Hussey, 2014). EHM affected horse that remains in standing posture may have good prognosis. However, horses with severe neurologic disease may take more than a year for complete recovery, although some horses may be left with permanent neurologic sequalae.

7 Factors affecting EHM

Mechanism behind EHM is poorly understood. Studies on the evaluation of the risk factors associated with the development of EHM have been performed in Europe and in North America. Various factors viz., season, age, breed, sex, immunological status and latency have been found to be associated with EHM. A study in the Netherlands revealed a strong association between season and outbreaks of EHV neurological disease with all outbreaks occurring between mid-November and mid-May. However, this season specificity has not been observed in all countries. Paillot et al. (2008) reported that neurological signs due to EHM were seen at an increased frequency in standard breeds, Hispanic breeds and draught breeds, with no cases of EHV-induced myeloencephalopathy in archetypical ponies, Haflinger, Fjord and Icelandic horses.

Experimental infection proved that older horses are more predisposed to the development of neurological disease as compared to young to young/middle aged horses. Adult horses may develop viremia 100 times higher than young horses and they are 8 times more likely to develop the disease (Allen, 2008). Latency by alpha herpes viruses is an important epidemiological strategy ensuring survival and spread within the natural host population (Whitley & Gnann, 1993). EHV1 latency has been demonstrated in lymphoid as well as in neural tissues (Baxi et al., 1995; Borchers et al., 1999). Following reactivation, latently infected carriers may shed the virus in their nasal secretion and also may result in EHM following invasion of nervous system (Allen & Timoney, 2007).

8 Laboratory Diagnoses

Laboratory diagnosis of EHM is currently based on at least one of the following criteria: clinical symptoms, cerebrospinal fluid examination, serological testing, virus isolation, molecular detection methods and post-mortem examination. Differential diagnosis should also be made from other viral cause of encephalitis, rabies, protozoal myeloencephalitis and non infectious conditions like neuritis of the cauda equina, central nervous system (CNS) trauma and different plant/chemical intoxications (Pusterla et al., 2009; Pusterla & Hussey, 2014). Horses presented with clinical symptoms as explained elsewhere may be suspected for EHM. An increased protein concentration and albumin quotient may be noticed in CSF of affected horses.

Serological examination suggesting a 4-fold or greater increase in serum antibody titer between acute and convalescent samples in the clinically affected horses, along with antibodies in CSF, is strongly suggestive of EHM (Friday et al., 2000; van Maanen et al., 2001). However, many horses with EHM do not exhibit a 4-fold rise in SN titer, since the antibody titers rise rapidly and may have peaked by the time neurological signs appear (Friday et al., 2000; van Maanen et al., 2001). Virus

isolation from nasal or nasopharyngeal swabs or buffy coat samples is considered as the 'gold standard' test for a laboratory diagnosis of EHV1 infection. However, EHM cases might not yield virus isolation, as virus shedding may stop by the time neurological signs appear (Pusterla et al., 2009).

Many of the published conventional PCR detection protocols (Ballagi- Pordany et al., 1990; Sharma et al., 1992; Wagner et al., 1992; Borchers & Slater, 1993; Kirisawa et al., 1993; Lawrence et al., 1994., Wang et al., 2007) are unable to differentiate between neuropathogenic and non-neuropathogenic viruses. Hence, PCR assays based on ORF30 followed by sequence analysis can be used to differentiate neuropathogenic and non-neuropathogenic EHV1 isolates (Nugent et al., 2006; Allen, 2007; Leutenegger et al., 2008; Pusterla & Hussey, 2014). Use of novel PCR platforms, such as real-time PCR assays based on ORF30 enable the differentiation of neuropathogenic and non-neuropathogenic viruses (Allen, 2007; Leutenegger et al., 2008). Single nucleotide polymorphism (SNP)-real-time PCR (Smith et al., 2012) and primer-probe energy transfer method (Malik et al., 2010) have been used for diagnosis of EHM. A SNP-based real-time PCR has been developed in our laboratory that is able to differentiate neuropathogenic and non-neuropathogenic EHV1 strains. Using this assay, we observed circulation of neuropathogenic EHV1 among Indian equine population (unpublished data).

9 Treatment of EHM

There is no specific treatment for EHM and the line of treatment is aimed at supportive medication to reduce CNS inflammation. Antiviral drugs for reducing viremia, non-steroidal anti-inflammatory drugs (NSAID) for countering inflammation and anti-thrombotic drugs for preventing clot formation are commonly used for treatment (Lunn et al., 2009; Pusterla & Hussey, 2014). Treatment with corticosteroids, such as prednisolone acetate or dexamethasone for 2 to 3 days, is frequently recommended for severely affected animals as their use could aid in reducing the incidence of vasculitis, thrombosis, and the resultant neural injury. Flunixin meglumine (nonsteroidal anti-inflammatory drug), which is commonly used for the treatment of CNS vasculitis can be used as these drugs suppress cellular interactions between infected lymphocytes and endothelial cells (Pusterla et al., 2009). Drugs like dimethyl sulfoxide, acetylsalicylic acid and pentoxifylline have also been used for thromboembolic events associated with vasculitis. Antiviral drugs such as acyclovir have been found effective in in-vitro studies, however, limited data is available on the in vivo efficacy of acyclovir. Administration of broad-spectrum antimicrobials is also found effective to combat the risk of development of cystitis in affected horses (Pusterla & Hussey, 2014).

10 Control of EHM

There is no specific method for prevention and control of EHM. However, routine management practices aimed at reducing the likelihood of introduction and dissemination of EHV1 infection can prevent EHM in herd. The control measures are mainly focused around quarantine and vaccination (Lunn et al., 2009; Pusterla et al., 2009). Affected or suspected horses must be removed from the stable immediately and placed in strict isolation. Once EHV1 infection is confirmed, horses should remain in strict quarantine until they are fully recovered and are asymptomatic for 21 days. Horses from farms experiencing EHM infection should be maintained in their existing stable and segregated from other horses. There should be total movement restriction of animals from such farms (Pusterla et al., 2009; Pusterla & Hussey, 2014). The currently used EHV1 vaccines are not able to provide protection against EHM. However, regular use of commercially available EHV1 vaccines enhances herd immunity, reduce viral shedding at the event of exposure and hence reduce EHM risk (Pusterla & Hussey, 2014).

11 Conclusions and future perspectives

The development of neurological disease due to EHV1 infection is likely to be multi-factorial. Potential horse-specific risk factors for EHM include advanced age, breed, post-exposure viraemic load, low cytotoxic-T lymphocyte precursors and environmental factors. Antemortem diagnosis of EHM relies mainly on real time-PCR detection of EHV1 in nasal secretions and blood. Although several vaccines are commercially available to prevent respiratory and abortigenic form of EHV1 infections, they do not provide protection from neurologic form of the disease. Even though there is a strong association between EHM and the G_{2254} mutation, this nucleotide substitution is not the only determinant of neurological disease. EHV1 isolates with A_{2254} genotypes have been associated with a number of cases of neurological disease. On the other hand, G_{2254} genotype EHV1 isolates have been recovered from horses with no evidence of neurological symptoms.

One of the possible reasons for this observation could be the fact that besides $A_{2254}\rightarrow G_{2254}$ substitution, other non-synonymous nucleotide substitutions in ORF30 region can also have an effect on the production of neurological disease by either enhancing/attenuating the capability of viral replication rates *in vivo*. Furthermore, DNA polymerase is only one out of six proteins involved in 'elongation complex' of DNA replication machinery Substitutions occurring in the ORF of any one of these proteins could have a considerable impact on viral replication rates, which will in turn have an effect on neuropathogenicity.

This is an area of research that needs further investigation. Comparative whole genome sequencing of neuropathogenic EHV1 strains from different geographical location might decipher other markers related to neuropathogenicity. There is also need to understand the role of host factors in the pathogenesis of EHM, including host immunopathological mechanisms in response to EHV1 infection and latency.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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