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Review Article

Equine Herpesviruses: a Brief Review

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ABSTRACT

Nine equine herpesviruses (EHV) have been known to infect equines. Equine herpesvirus type–1 (EHV1) is an important and ubiquitous viral pathogen of the horse and produces syndromes of respiratory disease, epidemic abortion and sporadic encephalomyelitis. This review article is focused on some aspects of EHV1 biology, its life cycle and pathogenicity in the natural host. Vaccination is one of the best options to fight EHV1 infection. Various strategies of vaccination that have been investigated and developed over the past decades will be presented in this review. Diagnosis is important to start specific treatment. The latest diagnostic techniques which were developed recently together with conventional will also be discussed.

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INTRODUCTION

Equine herpesviruses (EHVs) belong to the family *Herpesviridae*; a term derived from a latin word which means 'to creep'. The family is subdivided into three subfamilies (α β and γ) based on tissue tropism, pathogenicity and behavior in tissue culture (McGeoch et. al.,2000; Davison et. al.,2002). Nine species of EHV have been reported (Table 1). The species EHV1, EHV3, EHV4, EHV8 and EHV9 have been classified under the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae* of the order *Herpesvirales*. The species EHV2 and EHV5 have been put under a new genus *Percavirus*, subfamily *Gamaherpesvirinae*, family *Herpesviridae* of the order *Herpesvirales*. The EHV6 and EHV7 have been tentatively placed as species in the subfamily *Alphaherpesvirinae* and *Gammaherpesvirinae*, respectively (Davison at el., 2009). Only five of the nine herpes viruses (viz. EHV1, 2, 3, 4 and 5) have the ability to produce disease in horses. EHV3 is responsible for coital exanthema while EHV2 and 5 are not associated with a specific disease but may remain associated with upper respiratory tract diseases, inappetance, lymphadenopathy, immunosupression, keratoconjunctivitis, general malaise and poor performance (Thein et al. 1978; Plummer et al.1973; Belak et al. 1980). Both EHV1 and 4 are economically important viruses affecting the respiratory tracts of horses globally (Davison et. al., 2009; Roizman, 1996; Allen and Bryans, 1986; Brosnahan and Osterrieder, 2009). However, only EHV1 causes abortion and neurological disorders (Patel and Heldens, 2005; Lunn et. al., 2009). It was only in 1981 that the EHV1 and EHV4 were considered antigenically and genetically two distinct viruses, and not the same virus, based on DNA fingerprint analysis (Sabine et. al., 1981; Studdert et. al., 1981). This review will mainly focus EHV1 together with EHV4.

Table 1: Equine herpesviruses

Species	Sub family	Genus	Disease
Equine herpes virus 1	α	*Varicellovirus*,	Respiratory, Abortion, Neurological
Equine herpes virus 2	γ	*Percavirus*	NA
Equine herpes virus 3	α	*Varicellovirus*,	Coital exanthma
Equine herpes virus 4	α	*Varicellovirus*,	Respiratory
Equine herpes virus 5	γ	*Percavirus*	NA
Equine herpes virus 6	α	*Varicellovirus* (not approved)	Coital exanthma
Equine herpes virus 7	γ	Not Assigned	NA
Equine herpes virus 8	α	*Varicellovirus*,	Rhinitis
Equine herpes virus 9	α	*Varicellovirus*,	Gazelle and Equine neurological

NA= Not associated

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VIRUS STRUCTURE

The equine herpes virions with a diameter of ~150 nm have icosahedral nucleocapsid surrounded by a layer of globular tegument which in turn is enclosed in an envelope. Several glycoprotein peplomers are present on the viral envelope. The genome of equine herpes virions is made up of single linear dsDNA molecule with 57% G+C ratio. The genome size slightly varies between EHV1 (150 kbp) and EHV4 (145 kbp) (Darlington and Randall, 1963; Roizman et. al., 1992). Another feature of the genomes EHV1 and EHV4 is that they are composed of a unique long (U_L) region attached to a unique short (U_S) region. A set of indirect repeat sequences called internal and terminal sequences (IRS and TRS) flank the U_S (Figure 1) (Telford et. al., 1992, 1994). The U_L, U_S and IRS regions have 63, 9, and 4 genes, respectively, out of the total 76 genes reported in EHV1 and EHV4. Well–controlled expression of the genes divided into three distinct phases viz. Immediate Early (IE), Early (E), and Late (L) is observed (Caughman et. al., 1985, Albrecht et. al., 2005). Whole genome sequencing (Teleford et. al., 1992, 1998) of EHV1 and EHV4 indicates that the genome actually has 80 and 79 open reading frames (ORFs), respectively, due to duplication of some genes. Various proteins of EHV1 and EHV4 have been found to be identical at amino acid sequence homology in the range of 55% to 96% (Teleford et. al., 1998).

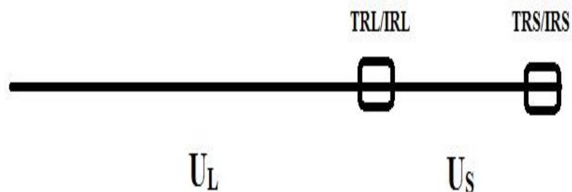

Figure 1: EHV1 genome: a unique long (U_L) region attached to a unique short (U_S) region. A set of indirect repeat sequences called internal and terminal sequences (IRS and TRS) flank the U_S.

PATHOGENESIS

Respiratory form

The binding of the EHV1 and EHV4 to heparan sulfate present on the surface of cell and subsequent interaction with a putative glycoprotein D receptor(s) leads to initiation of infection, (Azab and Osterrieder, 2012). Subsequent to attachment, entry of virus takes place either by receptor mediated endocytosis or direct fusion of the virus envelope with the plasma membrane. It has been observed that multiple endocytic pathways are utilized by EHV1 in different cell types to establish productive infection (El–Hasebe et. al., 2009). Equine brain microvascular endothelial cells are infected via caveolar endocytosis, while equine dermal cells involve energy– and pH–dependent endocytosis. After removal of the viral envelope following fusion, the naked virus particles start their replication in the nucleus after travelling through the cytoplasm. The integrity of the microtubule network and the minus–end microtubule motor protein, dynein plays an important role in the intracellular trafficking resulting in EHV1 infection (Frampton et. al., 2010).

After inhalation, the spread of EHV1 and EHV4 to regional lymph nodes occurs after their multiplication in the epithelium of respiratory tract (nasal cavities, pharynx, trachea, bronchi and bronchiole). Intranuclear inclusion bodies along with necrosis have been reported in respiratory epithelia and lymphoid germinal centres. A murine respiratory model has been used to study the pathogenesis of EHV1–induced respiratory disease in the horse (Awan et. al., 1991; Walker et. al., 1999). The role of proinflammatory beta chemokines in the pathogenesis of EHV1 have also been demonstrated (Smith et. al., 2000). Recently, pulmonary fibrosis in horses could experimentally be induced with the EHV5 (Williams mail et. al., 2013).

Abortion and Neonatal Disease

Abortion can be the result of exogenous or endogenous infection, i.e. reactivation of latent virus (Allen et. al., 1998). EHV1 virus after infecting respiratory tract spreads quickly throughout the body by invading leucocytes, lamina propria and endothelial cells of blood and lymphatic vessels due to a cell–to–cell spread of infectious virus from the respiratory epithelium.

Cell–associated viraemia is the result of movement of infected mononuclear cells from the regional lymph nodes into the circulation. The virus then cross the placenta, infect fetus and ultimately leads to abortion (Bryans et. al., 1969). EHV1 replicates in endothelial cells and induces thrombosis and ischaemia in the microcotyledons of the placenta. This was found to be the primary cause of abortion (Edington et. al., 1991; Smith et. al., 1992). However, not all EHV1 isolates have equal potential to cause abortion (Mumford et. al., 1994). Abortions induced by EHV1 don't affect the mare's subsequent reproductive efficiencies (Crabb et. al., 1996). Foetus may be born alive if infected in later stages of pregnancies, but soon after birth it dies because of respiratory distress, pneumonia and other respiratory complications (Crabb et. al., 1996).

Neurological Disease

EHV1 is non–neurotropic, even after intracerebral inoculation, in contrast to several other alphaherpesviruses (Jackson et. al., 1977; Prickett, 1969). Induction of myeloencephalitis reflects endotheliotropism but not neurotropism (Edington et. al., 1986; Jackson et. al., 1977; Patel et. al., 1982; Thein et. al., 1993; Whitwell and Blunden, 1992; Wilson, 1997). The minor blood vessels in the brain or spinal cord exhibit thrombosis and vasculitis due to infection of endothelial cells. However, multiplication of EHV1 strains in neurons and glial cells is observed after intracerebral inoculation of baby mice (Nowotny, 1987) resulting in neuronal latency in mice (Marshall and Field, 1997). Inbred mouse strains have proved to be very valuable models for studying the pathogenesis of EHV1 induced myeloencephalopathy in horses (Mori et. al., 2012). Recently, in suckling hamster model, it was demonstrated that EHV9 invade the brain via the trigeminal nerve besides the abducens, oculomotor, and facial nerves, which suggests the neuronal spread of neuropathogenic viruses to the brain via the eyes (Habashi et. al., 2013). Macroscopic lesions in the CNS include focal haemorrhage in parenchymal tissues of the brain and spinal cord which are not found frequently.

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Congestion, vasculitis, thrombosis and secondary ischemic degeneration are some of the significant histological lesions found in the CNS (Wilson, 1997). The requirement of horses as experimental animals to investigate EHV1 neuropathogenicity can be reduced with the use of equine respiratory explants for in vitro studies (Annelies et. al., 2010) with promising and reproducible results.

The neuropathogenicity of EHV1 strains is significantly associated with single nucleotide polymorphism ($A_{2254} \rightarrow G_{2254}$) in the open reading frame (ORF) 30, of the viral DNA polymerase, resulting in an amino acid variation from aspargine to aspartic acid (N/D_{752}) (Figure 2) (Nugent et. al.,2006; Van de Walle et. al.,2009). How EHV1 neuropathogenic phenotype is influenced by the DNA polymerase genotype requires further studies (Pronost et. al., 2010). The site directed mutagenesis has been applied to further investigate and confirm relationship between single nucleotide polymorphism in EHV1 polymerase and

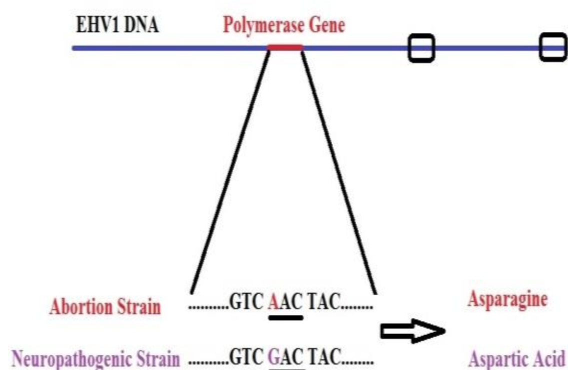

Figure 2: Single nucleotide polymorphism in EHV1 DNA polymerase gene: nucleotide substitution (A2254 to G2254) in polymerase gene leads to conversion of abortive strain to neuropathogenic strain

LATENCY

EHV1 after infection produce latent infection in over 50% cases in neuronal and lymphoid tissues (Pusterla et. al., 2012) and persists for the life time in the host (Foote et. al., 2006). Latent infection can be reactivated by stress, corticosteroids or drugs (Browning et. al., 1988; Nageshi et. al., 1992; Barrandeguy et. al., 2008). Prototype of alphaherpesviruses i.e. herpes simplex virus establish latent infection in neuronal tissues (Stevens, 1989; Stevens, 94). In contrast to it, EHV1 settles its latent infection in neuronal as well as lymphoid tissues (Edington et. al., 1985; Edington, 1994; Welch et. al., 1992). During latency entire viral genome persists in the infected cell but only its limited part transcribe in to latency associated transcripts (LATs), i.e. the only detectable RNA during latency. In EHV1 two LATs have been identified, derived from immediate early genes (gene 63 and 64) (Baxi et. al., 1995; Chester et. al., 1997; Borchers et. al., 1999). Targeting these LATs, latency can be detected by in situ hybridization, northern blotting, reverse transcriptase polymerase chain reaction (RT–PCR) or by real–time PCR (Stroop et. al., 1984; Stevens et. al., 1987; Pusterla et. al., 2009). Latency also has been identified as the absence of detectable expression of viral gene (ORF33) that encodes the late structural protein B (gB) (Allen et. al., 2008).

CLINICAL SIGNS AND SYMPTOMS

Respiratory Disease

The respiratory disease of equines can be caused by EHV1 as well as EHV4 (Allen et. al., 1986). Both viruses generally lead to mild or subclinical infection. The incubation period ranges from 2 to 10 days. The respiratory signs for EHV1 and EHV4 include high fever of 102 –107º F for 1–7 days, inappetence, depression, nasal discharge and coughing. The clinical signs are often mild or even absent after subsequent re–infection.

Abortion and neonatal Disease

EHV1 is a leading cause of abortion in horses worldwide. Incubation period is highly variable between nine days to four months (Allen et. al., 1998). Abortion usually occurs after 2–12 weeks of infection in late gestation (between 7 and 11 months of gestation). The infection doesn't cause damage to mare's reproductive tract, and she can conceive later. Mares if infected late in gestation period give birth to live foal but it can die within a few days after birth (Perkins et. al., 1999).

Encephalomyelitis

In recent years increased incidence of Equine herpesvirus myeloencephalopathy (EHM), has been observed by infection with some virulent strains of EHV1 leading to inflammation of the blood vessels that supply the brain and spinal cord (Henninger et. al.,2007). The neurological signs of Equine herpesvirus myeloencephalopathy (EHM) includes multifocal myeloencephalopathy, ischemic neuronal injury, hemorrhage andthrombosis (Wilson, 1997). The incubation period of encephalomyelitis is of 6–10 days. The onset of the neurologic signs is sudden and can vary from mild in–coordination and posterior paresis to severe posterior paralysis with recumbency, altered gaits, ataxia, staggering, inability to rise from the sitting position, loss of anal and bladder tone, and urinary incontinence. On rare occasions the paralysis may advance to quadriplegia and finally death of the animal (Studdert et. al., 2003, Goehring et. al., 2005).

TRANSMISSION

EHV1 is commonly referred to as the equine abortion virus. EHV4 is most common among foals and yearlings and, is also referred as equine rhinopneumonitis virus. The principal reservoir of infection for EHV is latently infected horses. It usually survives for less than 7 days in the environment, but has been detected for up to 35 days and is easily inactivated by heat and disinfectants. Equine herpesvirus spreads directly from horse–to–horse and indirectly by inanimate objects by coming in contact with nasal secretions and aborted fetuses or fetal fluids and aerosol from coughing horses. It is mainly transmitted by inhalation of the infected droplets or by the ingestion of material contaminated with nasal secretions, aborted fetuses, and placental membranes (Hebia et. al., 2007). Latent infection and its reactivation play an important role in the epidemiology of EHV1 abortion and neurologic disease (Figure 3).

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PREVENTION

Vaccination is an effective tool to prevent EHV1 infection together with good managing practices (Kapoor et. al., 2010). Successful vaccination against EHV1 require both humoral as well as cell mediated immune response in a balanced proportion. Various commercialized vaccines can be categorized in to live attenuated, inactivated, subunit, recombinant and DNA vaccines. However, inactivated EHV1 vaccine is main vaccine which provides the variable level of after experimental infection. In a recent study, ponies vaccinated twice with a conventional whole EHV1 inactivated vaccine adjuvanted with carbomer developed a strong humoral response (both CF and VN antibodies) after both immunizations (Minke et. al., 2006).

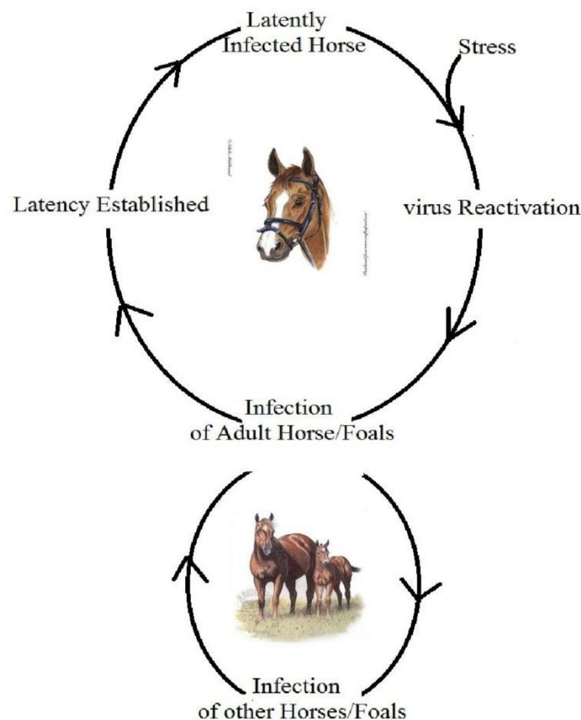

Figure 3: The transmission cycle of EHV1 in horses

Schedule of Vaccination

Two vaccinations one month apart are required to induce immunity, followed by boosters at every six months. Vaccination is recommended for foals over 3–5 months of age. It is best not to vaccinate horses when they are sick. To avoid EHV1 induced abortion, it is recommended to vaccinate pregnant mares at the 5th, 7th and 9th months of pregnancy.

INERT VACCINES

Inactivated whole EHV1 Vaccines and Sub–Unit Vaccines

EHV1 vaccines were first introduced in 1960. However, majority of the commercial vaccines are either inactivated whole virus vaccine or subunit vaccine. The major advantages of these vaccines are high degree of safety, as the virus replication and subsequent spread between hosts have been blocked. Viruses are usually inactivated by formaldehyde or β– propiolactone. Nowadays, inactivated whole EHV1 vaccines are widely used. In foals, vaccination significantly reduced symptoms of disease and the time–course of both cell–associated viremia and shedding of virus

Despite an extensive use of inactivated virus vaccines in many countries over decades, abortion storms are still being reported (van Maanen et. al., 2000). Presently, whole virus inactivated vaccines have been replaced by subunit or split vaccines. The split or subunit vaccine is produced by disintegration of purified virion particles usually by chemical treatment. They generally induce similar adverse reactions because they contain the same constituents as inactivated whole virus vaccines. Due to the large number of proteins expressed by EHV1, the selection of relevant proteins for subunit EHV1 vaccines tends to be difficult. Glycoproteins of EHV1 are essential for cell receptor recognition and the entry into target cells. Glycoproteins B and C contain antibody epitopes strongly recognised by the immune system of horses infected or vaccinated with EHV1, and are therefore candidates for inclusion in sub–unit vaccines against EHV1 (Tewari et. al.,1995). Recombinant baculoviruses having gD and gH genes induce serum antibodies to EHV1 (Fuentealba et. al., 2014). Similar results have been obtained with baculovirus recombinant gC and gB (Kukreja et. al., 1998; Tewari et. al., 1995).

Immunostimulating Complex–Based Vaccines

Immunizations with proteins generally require the presence of strong adjuvants to stimulate an immune response. The *Quillaia* saponin (Quil A) are generally used as adjuvant and membrane protein/antigens are usually integrated in immuno–stimulating complexes (ISCOM™) or mixed with Iscomatrix™ to improve their antigenicity. ISCOMs and Iscomatrix™ are not fusogenic but can associate with intracellular lipid membranes in the cytoplasm and vesicular compartments (Bungener et. al., 2002).

An experimental sub–unit vaccine containing glycoproteins gp2, gp10, gB, gC, gD and gM, of EHV1 and adjuvant Iscomatrix™ showed antibody response with virus neutralization (VN) activity in hamsters (Cook et. al., 1990). A similar vaccine composed of inactivated Sf9 insect cells infected by a recombinant baculovirus coding for EHV1 gD and mixed with the adjuvant Iscomatrix™ was administered to ponies, pregnant mares and foals via the intramuscular route (Foote et. al., 2005). Vaccinated ponies developed a virus–specific antibody (having IgGa, IgGb, IgG (T) and IgA antibodies) response with neutralizing activity (Weerasinghe et. al., 2006) which was similar to the response induced by inactivated whole virus EHV1 vaccine.

Mares and foals vaccinated twice with a sub–unit vaccine containing both gB and gD mixed with the Iscomatrix™ adjuvant have shown reduced virus shedding. However, cell–associated viremia was not affected by vaccination (Foote et. al., 2006).

DNA Vaccines

DNA vaccine consists of plasmids expressing gene of whole antigenic proteins or simply epitopes of these proteins. These antigens are directly expressed *in vivo* in the target host cell and ultimately result in stimulation of both humoral as well as cellular immune responses. DNA vaccines can also be adjuvanted by the insertion of genes

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coding for co-stimulatory molecules (e.g. cytokines), theoretically allow a more controlled modulation of the immunity than the one induced by conventional adjuvant vaccines. DNA vaccines can be lyophilized; hence they have good stability and long-term storage. A DNA vaccine encoding gD has been prepared which elicit both cell-mediated as well as humoral immune responses (Ruitenberg et. al., 1999). An increased level of gD-specific antibody was observed in horses after intramuscular administration of DNA vaccine encoding gD (Ruitenberg et. al., 2000). The DNA vaccine containing coding genes of gB, gC and gD glycoproteins of EHV1 has been tested. DNA constructs were administrated alone, in combination with adjuvants (i.e. aluminum phosphate or carbopol), or complexed with the cationic lipid DMRIE-DOPE in the presence of a DNA plasmid coding for granulocyte-macrophage colony stimulating factor (GMCSF). The amount of VN antibody was increased in ponies vaccinated with the DNA/DMRIE-DOPE vaccine, and the duration of nasopharyngeal virus excretion after experimental infection with EHV1 was reduced following immunization with a DNA vaccine adjuvanted with aluminium phosphate (Minke et. al., 2006). It is clear that DNA vaccination efficiently stimulates an EHV1 specific antibody response in the horse but so far no cellular immune responses have been studied. Therefore, the protection afforded by this type of vaccine needs further investigation.

Non Infectious EHV1 L-Particles

The generation of non-infectious virion-related particles in cells shortly after infection with EHV1 has been carried out (McLauchlan and Rixon, 1992). They are comparatively less known type of virus-like particles formed by tegument proteins surrounded by an envelope and lack the internal capsid protein and viral nucleic acid. These L-particles do not contain viral DNA which makes them noninfectious. However, their capability to transport viral envelope proteins to target cells remains unaffected, and thus has no effect on their biological competence (Pardoe and Dargan, 2002). L-particles possess an intact envelope membrane with all immunogenic glycoproteins so they are expected to stimulate an immune response towards the viral glycoproteins similar to that during the natural viral infection. As a result, they have been identified as potential immunising agents. They are named light-particles (L-particles) because of their ability to be separated into a low density band on Ficoll gradients (Dargan and Subak-Sharpe, 1997). L-particles could support initiation of viral replication at the early stage of infection and/or may serve as decoys for the immune response. However, the exact role of L-particles in infection has not been established. In specific-pathogen-free (SPF) foals, immunization with EHV1 L-particles induced a CF antibody response. Clinical signs of disease, virus shedding and cell-associated viremia were milder in immunized ponies when compared with unvaccinated animals.

A new type of virus-related particle, called pre-viral DNA replication enveloped particles (PREPs), has been developed (Dargan et. al., 1995). The relative protein composition and structure of these PREPs is different from L-particles, though these two have similar morphology.

LIVE EHV1 VACCINES

Live vaccine contains live microorganisms such as live attenuated or a genetically modified organisms, or heterologous organism used as vector for the gene coding pathogen's antigen.

Live Attenuated Vaccines

The immunization with live attenuated virus is very similar to natural infection. The intranasal or intramuscular inoculation of a live attenuated/modified EHV1 is an attractive approach for the vaccination against EHV1. However, the risk of reversion to virulence remains a concern for this strategy of vaccination. Two principal types of EHV1 mutants have been derived and used as live attenuated EHV1 vaccines, namely thymidine kinase negative (TK-) and temperature sensitive (Ts) mutants. Their virulence and ability to establish latency is reduced in the horse while immunogenicity and stability is retained. Immunization of EHV1 unprimed ponies with TK- EHV1 by intramuscular or intravenous injection did not induce any sign of disease occurrence. However, when vaccinated ponies were challenged with EHV1 virus, an anamnestic VN antibody response was observed. The ponies immunized with TK- EHV1 vaccine also showed less clinical signs of disease and live virus shedding to environment (Cornick et. al., 1990). However, live attenuated vaccine for EHV1 may not always produce optimal immune response. The intranasal administration of a live attenuated EHV1 vaccine (Rhinomune, Pfizer) failed to induce mucosal antibodies, which could explain the failure of attenuated EHV1 vaccines to prevent virus shedding. It was speculated that the attenuation may have reduced the virus's mucosal immunogenicity (Breathnach et. al., 2001).

It was observed that mutant EHV1 viruses deficient in the whole IR (vL11ΔIR) produced plaques of small size and showed belated growth kinetics (Ahn et. al., 2011). The CBA mice intranasally infected demonstrated increased survival rate and decreased viral titre in the lungs compared to mice infected with parental or revertant virus.

Poxvirus-Based Vector Vaccine for EHV1

In live vector vaccine the epitope specific gene of pathogens are inserted in to genome of nonpathogenic infectious viruses through modern biotechnology tools. These viruses will express the epitope specific proteins in the host cell and will induce both humoral as well as cell mediated immune response. For such vaccine preparation recombinant poxviruses are generally used (Moss, 1996) because they are genetically stable and can accommodate relatively larger size inserts.

Several recombinant vaccines for horses have been developed from recombinant pox viruses such as vaccinia virus and avipoxvirus (Minke et. al., 2006). A new recombinant vaccinia virus derived (NYVAC strain) vaccine specific to IE protein (CTL target protein), has been introduced for vaccination in horses. The EHV1 vaccines derived from vaccinia virus can stimulate CMI in the horse and therefore induce protective immunity against the disease (Paillot et. al., 2006).

DIAGNOSIS

A number of diagnostic techniques based on microbiology and modern molecular biology tools have been used for

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diagnosis of EHV1. However, the 'gold standard' test for EHV1 infection diagnosis remains virus isolation. For estimation of EHV5 load in lung, the broncho–alveolar lavage is considered as good clinical sample (Marenzoni et. al., 2011). The EHV1 specific antigen and nucleic acid was detected in the trophoblasts of fetal membranes in spontaneous cases of equine abortion (Szerdi et. al., 2003).

The serum neutralization (SN) assay is traditional method for detection of EHV1 antibodies. However, it is unable to accurately differentiate the infected animals from vaccinated animals. However, in some experimental studies, co–relation of protection with SN titers has been reported (Goehring et. al., 2010; Goodman et. al., 2006). A further difficulty is the cross–reaction of EHV1 and EHV4 in SN assay (Hartley et. al., 2005).

A number of PCR based assays have been developed to target different genes of EHV1 such as thymidine kinase (TK) gene (Carvalho et. al.,2000) and various glycoprotein genes such as B (Borchers and Slater, 1993), C (Galosi et. al.,2001), D (Galosi et. al.,2001) and H (Varrasso et. al.,2001) for correct diagnosis. However, these techniques have some serious limitations in terms of being cumbersome and carry increased risk of cross contamination resulting from manipulation of amplified PCR product. However, few of these assays are either semi–nested or nested PCRs or used in combination with Southern blotting technique (Borchers and Slater, 1993; O'Keefe et. al., 1991).

Now–a–days, the real–time PCR has become a very valuable diagnostic tool in virology (Mackay et. al., 2002). The technique utilizes the combined activity of specific hybridization of a fluorogenic probe to a target gene and 5' exonuclease activity of Taq DNA polymerase. The target nucleic acid sequence is amplified and simultaneously Taq hydrolyses the bound dual–labeled probe. This in turn shows fluorescence which is detected and recorded by software and plotted as a graph (Livak et. al., 1995; Heid et. al., 1996). The real–time PCR assay is very specific, fast, sensitive, and effective in diagnosis of viral diseases. It also has the advantage of low risk of cross–contamination over conventional PCR.

The glycoprotein B gene specific multiplex real–time PCR has the potential to distinguish EHV1 and EHV4 (Diallo et. al., 2007). To each equine herpes virus, specific primers and probes were designed and used in monoplex or multiplex PCR. The EHV1 and EHV4, specific minor–groove binding probes (MGB^{TM}) labelled with 6–carboxyfluorescein (FAM^{TM}) and VIC^{R} probes respectively are commonly used for detection.

A real–time PCR assay using allelic discrimination (E2) to distinguish between neuropathogenic and non–neuropathogenic strains of EHV1 has been reported (Allen, 2007). It was observed that the neuropathogenic phenotype of EHV1 was associated with a single–nucleotide polymorphism (A2254 or G2254) in open reading frame 30 (ORF30). Undoubtly this real– time PCR assay can differentiate neuropathogenic and non–neuropathogenic strains. However, it lacks sensitivity for routine viral nucleic acid detection from various clinical specimens. Similarly, the development of ORF30 region specific, allelic discrimination (E_1) EHV1 real–time PCR test has been reported (Smith et. al., 2012).

Moreover, nasal swabs and blood samples used for rapid quantitative PCR testing have become standard practice for the diagnosis of active infections of EHV1 (Perkins et. al., 2008).

CONCLUSION AND FUTURE DIRECTIONS

Among the nine equine herpes virus types isolated from equines, the equine herpesvirus 1 (EHV1) and equine herpes virus 4 (EHV4) are responsible for significant economic losses to the equine industry globally. The infection with EHV1 can cause abortion, neonatal foal mortality, paralysis, and respiratory disease; while respiratory disease is the predominant outcome of EHV4 infection. The incidence of EHV1 induced equine herpesvirus myeloencephalopathy (EHM) has increased throughout the world during last decade. A single nucleotide polymorphism in EHV1 polymerase gene has been correlated with EHM, which may provide a selective advantage to neuropathogenic strain. The identification of factors playing a role in possible emergence of neuropathogenic strain needs to be investigated. The other reason of EHM could be reactivation/ recrudescence of latent EHV1 infection. Latency and reactivation are the key features of EHV infection which indicates the survival strategy of virus during course of evolution. However, our knowledge about EHV latency is quite scanty and it remains an underexplored area. Isolation of equine herpes viruses from new host species and new locations indicates the dynamic epidemiological picture of EHVs. The role of cell–associated viremia, latency and immune evasion in pathogenesis and epidemiology need further studies which in turn will help in devising control strategies.

Equine herpesvirus infection is difficult to clinically differentiate from other causes of equine respiratory disease, such as equine influenza, solely on the basis of clinical signs. Definitive diagnosis is determined by PCR or virus isolation from blood and the samples obtained from nose and throat. Suspected cases of EHV1 abortion can be confirmed by virus isolation, PCR and characteristic gross and microscopic lesions in the aborted foetus. Diagnostic techniques such as immunohistochemistry and PCR have diagnosed the cases of foetus negative, placenta–positive EHV associated abortion. Serological testing of mares after abortion shows little diagnostic value. Real–time PCR has shown its usefulness in diagnosing suspected cases of EHM. Also, there is need of a faster test to distinguish between the neurological strain and the non–neurological strain.

Gaps exist in our understanding of the correlates of protective immunity against EHV1. Vaccination against EHV is widely used especially among racehorses and brood mares, which helps in reducing virus shedding and duration of viraemia. None of current commercial vaccines is able to consistently prevent infection in a vaccinated animal or provide complete protection against diseases associated with EHV1. Currently used EHV vaccines have been shown to decrease clinical signs only in respiratory and abortive form of EHV infection. None of the current commercial vaccines gives protection against neurological form of the disease. There is a need to develop effective EHV1 vaccines that induce both mucosal and systemic cellular as well as humoral immunity in the horses along with a safe and efficient delivery route.

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