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Equine Herpes Virus Type 4 Pathogenesis, Diagnosis Prevention and Treatment: A Review

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ABSTRACT
Equine Herpes Virus type 4 (EHV-4) is a dsDNA alpha herpes virus which belongs to family Herpesviridae. It is a very important respiratory pathogen that causes outbreaks of respiratory disease in horses termed as Equine Rhinopneumonitis and occasionally causes abortions and neurologic disease. The respiratory disease caused by Equine Herpes Virus type 4 adversely affects racing performance of horses and also make them susceptible to other respiratory pathogens like strangles. Most damage occur after secondary bacterial infections. So rapid identification and diagnosis of disease is very important to prevent its spread and to stop the outbreak. An early diagnosis also helps to provide quick treatment to the infected horse and reduce the possibility of long term complications caused by secondary infections. Diagnosis of EHV-4 infection can be done by various methods such as VNT, Virus isolation test, CFT, PCR assay, ELISA and sequencing. Out of these methods PCR based assays are widely used for the rapid and more sensitive detection of EHV-4 and ELISA is used for the screening of anti-EHV1 and anti-EHV4 antibodies in the serum of infected horses. The present study was done to diagnose Equine Herpes Virus type 4 infection in horses using PCR technique and its differentiation from EHV-1 serologically. DNA was extracted from the nasal swab samples taken from diseased horses and was subjected to PCR for detection of Equine Herpes Virus type 4 DNA in the samples. Equine Herpes Virus type 4 infection and showed a single concise band of 507bp. PCR assay has been proved as a rapid, effective & highly sensitive diagnostic test for EHV-4 infection.

KEYWORDS:EQUINES, HERPESVIRUS, ETIOLOGY, EPIDEMIOLOGY, PATHOGENESIS, DIAGNOSIS

INTRODUCTION

The EHVs are most infective pathogens of all members of the equidae family that cause severe economic losses to horse industry worldwide. Among all Herpesviruses, EHV type 4 is one of the major respiratory pathogen of equines that causes acute respiratory disease in horses termed as Equine Rhinopneumonitis and occasionally also causes abortion or neurologic disease. It belongs to the family Herpesviridae, subfamily Alpha herpes virinae & genus Varicello virus.
EHV-4 has a linear, ds and type 'D' DNA genome of about 145 kbp having 76 genes which code for

different proteins. Virions consist of a core, capsid, tegument and envelope. The corecomprises dsDNA genome enclosed within an icosahedral capsid which is sheathed with a protein layer termed as tegument and a bilayer lipid membrane called as envelope. The lipid envelope contains several integral viral glycoproteins which play crucial role in the disease pathogenesis by involving in infection process.

EHV-4 infection is enzootic in Indian domestic horse population. It is responsible for upper respiratory tract disease in foals, weanlings and yearlings characterized by short incubation period of less than one day and symptoms include fever, coughing, profuse serous nasal discharge that later become mucopurulent, lethargy, anorexia and variable enlargement of mandibular lymph nodes. Fever can last up to 1-4 days, sometimes a second spike can occur approximately one week after the primary pyrexia. Respiratory illness caused by Equine Herpes Virus type 4 adversely affects racing performance of diseased horses and also make them susceptible to other respiratory pathogens like strangles. Most damage occur after secondary bacterial infections.

It is highly infectious & transmit rapidly via direct and indirect contact with infectious nasal secretion, inhalation of droplets from coughing of infected horses and ingestion of contaminated feed. Virus can be viable for several weeks in the environment after shedding bythe horse. Lifelong latency and reactivation after primary infection are the major factors of epidemiology of EHV-4 infection. The re-infection can occur after 3-4 months but clinical signs are mild or may be absent after re-infection. After repeated illness by EHV-4, a short term protection has been shown against the virus. Rapid diagnosis helps to provide quick treatment to the infected horse and reduce the possibility of long term complications caused by secondary bacterial infections.

REVIEW OF LITERATURE

History and Taxonomy

Diseases caused by EHVs leads to major economic losses to the equine industry. Up to now, nine types of equid herpesviruses (EHV-1 to -9) have been identified, of which six related to the Alpha herpesvirinae subfamily (EHV type 1,3,4,6,8,9) and the other three (EHV type 2,5,7) are the member of Gamma herpesvirinae subfamily (Davison et al., 2009). Equine is the common host to EHV type 1,2,3,4,5 (Patel and Heldens, 2005). Among all, Equine Herpes Virus type 1 & 4 are considered as the most important pathogens of Equines. EHV-4 was recovered from the upper respiratory tract of the equines of about all ages (Matsumura et al., 1992).

Equine Herpes Virus type 1 & 4 are closely related to each other. Before 1981, both were considered subtypes of same virus Equine Herpes Virus type 1 (Studdert et al., 1981). Until then EHV1 subtype 1 was called equine abortion virus and subtype 2 was called equine rhinopneumonitis virus universally (Crabb and Studdert, 1995). The differences in the pathogenesis and biological behaviour between both subtypes were recorded (Studdert and Blackney, 1979). Later on restriction enzyme analysis was carried out and differentiation wasdone between the subtypes of EHV-1, on the basis of differences in the DNA profile of the two subtypes (Sabine et al., 1981). In 1988, the ICTV of Viruses designated the two subtypes of EHV type 1 as EHV type1and 4 (Roizman et al., 1992).

Structure

EHV-4 has a linear ds type ‘D’ DNA genome of about 145-kbp (Roziman et al., 1992). The genome is distributed in a unique long and short region, the unique short region is bracketed by a pair of inverted

sequences called the internal & terminal repeats (Telford et al., 1992).The genome contains 76 genes which code for different proteins (Telford et al., 1998), from whichthree gene (64,65,66) are present in duplicate which results in 79 open reading frames.

Virions consist of a core, capsid, tegument and envelope. The core comprises dsDNA genomeenclosed within an icosahedral capsid which is sheathed in a protein layer termed as teguments and a bilayer lipid membrane called as envelope. The lipid envelope contains several integral viral glycoproteins which play an crucial role in disease pathogenesis & are involved in infection process i.e. adsorption, replication and transmission. (Baker et al., Davison, 2002; Pailot et al., 2008; Crabb & Studdert, 1990; Molinkova, 2012; Oladunni et al., 2019).

Eleven envelope glycoproteins are present on EHV-1 & 4 that function as viral immunogens. (Molinkova, 2012; Slater, 2007; Von Einem et al., 2007). For this reason, the identification and study of immunological components is very important for effective intervention against the disease (Patel & Haldens, 2005). Theseenvelope glycoproteins are involved in virus-host interactions as mediation of the virus entry in the host cell via adsorption, penetration & cell fusion (Patel & Haldens, 2005; Damiani et al., 2000; Slater, 2007; Maanen, 2002; Crabb & Studdert, 1990).The glycoprotein B, D, H, K and L are crucial for the virus replication and the glycoprotein C, E , I, G, M and gp300 are non-essential for replication (Patel & Haldens, 2005; Molinkova, 2012; Slater, 2007). Whereas these envelope glycoproteins which are not essential for viral replication play an crucial role in the virulence (Paillot et al., 2008; Damiani et al., 2000). Glycoprotein G (Gg) function as chemokine binding protein and expressed early in the infection pathway. It blocks the interaction of chemokines with their specific receptors, which helps the virus in evasion and suppression of immune system (Van De Walle et al., 2007). Equine Herpes Virus type 4 glycoprotein E (gE) consists of 548 amino acids having molecular weight of 95kDa approximately. (Damiani et al., 2000). It forms a heterodimer in association with glycoproteinI (gI) which is helps in cell to cell spread of virus (Slater, 2013).

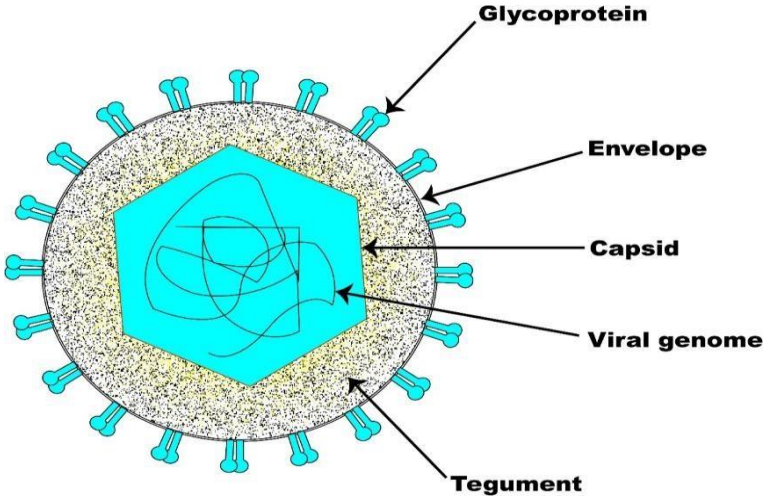

Fig.1. The structure of the Equine Herpes Virus. The virion consists of a core, capsid, tegumentand envelope enclosing the dDNA genome. The lipid envelope contains several integral viral glycoproteins. (Oladunni et al., 2019).

Pathogenesis

After attachment to the host cell, virus penetrate the cell by endocytosis or phagocytosis and release the tegument proteins and nucleocapsid into the cell (Frampton et al., 2007). EHV-4 enter in the epithelial cells of URT by an endocytic pathway (Azab et al., 2013). Equine Herpes Virus type 4 has been isolated from URT of the diseased equines (Allen and Bryans, 1986).

Glycoprotein G (gG) expressed by EHV-4 function as chemokine binding protein (Van de walle et al., 2007). Chemokines are crucial for modulation of leucocyte movement during innate & adaptive immune responses and the inability of Equine Herpes Virus type 4 glycoprotein G to modify chemokines was thought to be one of the reasons for restriction of Equine Herpes Virus type 4 infection to the upper respiratory tract (Osterrieder and Van de Walle, 2010). The ability of causing infection to different cells of Equine Herpes Virus type-4 in vitro was depend on glycoprotein D (Azab and Osterrieder, 2012). Equine Herpes Virus type4 has the inability to infect PBMCs in vitro and its entry into the PBMCs was not inhibited by blocking MHC-1 (Azab and Osterrieder, 2012). The study showed that unknown receptors were involved in the entry of EHV-4 into PBMCs & the complete blockade with antibodies is not possible due to very high number of available MHC-1 receptors.

Equine Herpes Virus type 4 is mostly associated with the respiratory disease called as equine rhinopneumonitis (Patel and Heldens, 2005). It is responsible for URT disease in foals, weanlings & yearlings. The spread of infection occur by direct contact, aerosols inhalation, nasal secretion & consumption of contaminated feed (Garre et al., 2009). After initial infection the virus can shed in nasal droplets for up to 3 weeks. Virus can be viable for several weeks in the environment after shedding by the infected horse.

Lifelong latency and reactivation after primary infection are the major factors of epidemiology of EHV-4 infection. The re-infection can occur after 3-4 months but clinical signs are mild or even negligible after re-infection. Respiratory illness caused by EHV-4 badly affects the racing performance of horses and make them susceptible to other respiratory pathogens like strangles. Most damage occur after secondary bacterial infections.

The symptoms of acute respiratory diseases due to EHV-4 are characterised by fever, anorexia, nasal discharge, pharyngitis, enlarged submandibular lymph nodes & ocular discharge occasionally (Patel and Heldens, 2005; Constable et al., 2017).Fever can last up to 1-4 days, sometimes a second spike can occur approximately one week after the primary pyrexia.

EHV-4 is not frequently related with equine abortions & paresis (Verheyan et al., 1998; Meyer et al., 1987). The cases of abortions due to EHV type 4 was <1% between 1983 and 1992 in Kentucky, USA (Ostlund, 1993) whereas in England between 1987-93 it was 16% (Whitwell et al., 1995). In another study of EHV-1/4 induced abortions, EHV-4 was responsible for 4% abortion cases out of 254 (Van et al., 2002). Blunden et al., 1995 examined the vascular lesions in a foal caused by EHV type 4. The study showed that the replication of EHV type 4 occurred in vascular endothelial cells suggestive of vascular basis of pathogenesis of EHV-4 induced abortions are identical to that of abortions caused by EHV-1(Smith et al., 1992; Edington et al., 1991). The severity of clinical disease caused by EHV-4 can be effected by the host & environmental factors (Balasuriya et al., 2015).

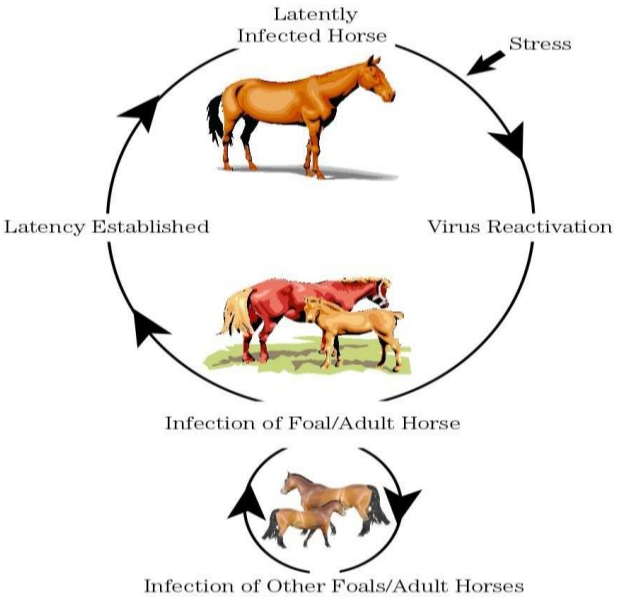

Fig.2. Life Cycle of EHV- 4

The capability of being latent, a state in which horses carry virus in an asymptomatic form forlong time, is one of the most powerful tool of this virus (Slater, 2007; Ostlound, 1993). During latency, the linear genome of the virus adopts a transcriptionally repressed circular configuration associated with non-acetylated histone and translocates to the nuclei (Gulati et al., 2015; Paillot et al., 2008). Latent stage gives protection to the virus from the antiviral effectsof the Tc- cells & neutralizing abs (Balasuriya et al., 2015; Reed & Toribio, 2004). Latency after primary infection plays an crucial role in the survival and spread of infection in host population (Whitley and Gnann, 1993).

Equine Herpes Virus type 4 latency has been showed in lymphoid tissues, peripheral leucocytes and in brain tissues (Gibson et al., 1992; Patel & Haldens, 2005; Edington et al., 1994; Baxi etal., 1995; Gulati et al., 2015; Baxi et al., 1995). Virus shedding was reported for reactivated Equine Herpes Virus type 4, upon corticosteroid treatment to the field infected horses (Browning et al., 1988). The reactivated virus can be transferred to other horses and can cause disease .Temporary cross protection has been shown against the disease after repeated infection with EHV-4. The re-infection can occur after three to four months but clinical signs were mildor even absent after re-infection (Allen and Bryans, 1986).

Epidemiology and Diagnosis

EHV-4 infection is indigenous in equine population globally (Allen et al., 1999; Allen and Bryans, 1986). Equine Herpes Virus type-4 is mainly related with the respiratory illness designated as equine rhino pneumonitis. The occurence of Equine Herpes Virus infection was 16.60% in south Gujrat, India (Vala et al., 2021). The rate of abortions due to Equine Herpes Virus type 4 was <1% between 1983 and 1992 in Kentucky, USA (Ostlund, 1993) whereas in England it was up to 16% between 1987 to 1993 (Whitwell et al., 1995). Backdated testing of samples collected from adult horses between 1967-74 & 1993 showed 100% positivity for Equine Herpes Virus type-4 Abs (Crabb and Studdert, 1995). The study showed that the prevalence of Equine Herpes Virus type-4 infection in adult thoroughbred population was veryhigh before the introduction of vaccination. In a study (Gilkerson et al., 1999b) >99%

of mares & foals were tested positive for Equine Herpes Virus type 4 antibody. During 1979-1990 a study was done on respiratory disease in racehorses in Japan showed that infection of EHV-4 occurs throughout of the year in horses of all ages while EHV-1 infection occurred mainly in winter season (Matsumura et al., 1992).

Diagnosis of EHV-4 infection can be done by different methods such as Virus neutralization test, Complement fixation test, qPCR, ELISA & sequencing. Serological tests can also be usedto identify past infections and vaccination status of animals. Nowadays, PCR based assay have been widely used for a rapid, more specific viral diagnosis and quantification of virus titre. PCR allows the analysis of a short sequence of DNA by rapidly amplifying a specified fragmentof DNA. Because of its high sensitivity and specificity, Polymerase Chain Reaction is most widely employed technique for the diagnosis of EHV-4 infection. ELISA is also a commonly used immunoassay technique in the diagnosis of EHV-4 in horses. It is used to detect antibodies in the serum samples. Different samples such as nasal swabs, serum and aborted foetus tissues can be used for the diagnosis of EHV-4 infection in suspected animals. However ELISA has less sensitivity than virus isolation but PCR is highly sensitive as compared to ELISA and virusisolation.

EHV-4 and EHV-1 infection cause similar respiratory symptoms, however EHV-1 in addition is a primary cause of abortions and neurological disease in equines. It is difficult to differentially diagnose them clinically and serodiagnosis of these viruses is also complicated due to the extensive antigenic cross-reactivity. In 1990s, a viral glycoprotein G was identifiedto evoke type- specific immunoglobulins. Now differentiation between EHV-4 and EHV-1 is made possible due to the development of a type-specific ELISA which detects Abs to type specific epitopes of glycoprotein G of both the viruses. DNA fingerprinting can also be used for the differentiation of these viruses but it is a laborious technique, so it is not used routinely.While using virus neutralization test and complement fixation test, serum samples should be tested in duplicate for both EHV Type 1 & 4 to minimize the risk of false -ve results.

Various tests such as serological test methods & different PCR assays are used to diagnose the virus (Crabb and Studdert, 1995). PCR allows the identification of the virus by rapidly amplifying a specific segment of DNA using specific primers. qPCR is a rapid & highly sensitive technique used for the diagnosis of disease and to quantify viral titre (Milic et al., 2018). Different types of samples like nasal swabs, blood or tissues can be used with these molecular techniques (Mannen, 2002).

EHV-1 & EHV-4 has been detected by serological methods (Yasunage et al., 1998, Singh et al., 1999) & virus isolation from nasal secretions blood and aborted foetus tissues (Taouji et al., 2002; Barrandegry et al., 1999).The interpretation of data from serological surveys of EHV-4 and EHV-1 was complicated due to extensive antigenic similarity and lack of type-specific antibodies until early 1990s (Crabb and Studdert, 1993). The envelope glycoproteins which are engaged in DNA replication & packaging and formation of capsid show the closest homologybetween EHV-1 & 4. Differences were noticed in the 3' end of gene seventy which encodes .

In early 1990s viral gG was recognized to evoke type-specific Ab. By comparing, 58% similarity was found between the coding sequences of EHV-4 gG and EHV-1 gG, however, the peptide regions comprising 287 to 382 amino acids residues in EHV-4 gG and 288 to 350 in EHV-1 gG showed only 21% similarity (Crabb & Studdert, 1993). These findings suggested that this region may be used for differentiating these viruses.

Nowadays, differentiation between EHV-4 and EHV-1 is made possible due to the progression of a type-specific ELISA detecting Ab to isotopes of glycoprotein G of both the viruses (Van Mannen, 2002; Lang et al., 2013; Balasuryia et al., 2015). ELISA can be used both for detection of antigen and antibodies (Dutta et al., 1983; Singh et al., 1994a). A type-specific antibody test was used for the 1st time by Crabb and Studdert, in samples of TB horses, all equines were found +Ve for Equine Herpes Virus type 4 infection and 9 percent were +Ve for EHV type 1 infection (Crabb and Studdert, 1993). In one more study the seropositivity of Equine Herpes Virus type 4 specific antibodies was found 100% again and seropositivity of Equine Herpes Virus type 1 Abs was found 30% (Crabb et al., 1995). The prevalence of Equine Herpes Virus type 4 infection in horses in Turkey was examined by (Ataseven et al., 2009) using ELISA and multiplex PCR. The results demonstrated that 81.7 percent of the tested horses were seropositive for EHV type 4. EHV type 4 DNA was found in the nasal swabs samples taken from the equines showing infection symptoms (Ataseven et al., 2009). Diagnosis of EHV-4 infection was done using PCR in symptomatic horses of south Gujarat, India (Vala et al., 2020).

PCR has been proved to be a better alternative and has shown greater sensitivity than virus isolation test for diagnosis & differentiation of Equine Herpes Virus type 1 & 4 (Borchers and slater, 1993). Recently molecular analysis & gene sequencing have disclosed genetic variance between EHV-1 & EHV-4 (Harless and Pusterla., 2006).

PREVENTION AND TREATMENT

During an outbreak of Equine Herpes Virus type 4 respiratory disease, the infected horses should be secluded for at least three weeks and proper disinfection of place should be done to prevent spread of infection to other horses in close proximity. Inactivated combined EHV- 1/EHV-4 vaccines are also available commercially. Proper nutrition should be given to the infected animals and stress should be avoided for fast recovery (Allen and Bryans, 1986). Secondary bacterial infections can be prevented by giving antibiotics & fever can be treated by giving antipyretics to the affected animals (Mannen, 2002; Slater, 2007).

Different types of vaccines like Inactivated vaccine, Modified live virus vaccine , Subunit & Recombinant vaccine and DNA vaccines are available which can help in reducing severity of respiratory infection symptoms and also reduce shedding of virus from nasal mucosa of infected horses. These vaccines do not give complete immunity against the virus but help in the prevention of systemic dissemination and reactivation of virus (Paillot et al., 2008)

CONCLUSION

Equine Herpes Virus Type 4 is the most significant respiratory pathogen which causes acute respiratory disease of horses termed as Equine Rhinopneumonitis and occasionally causes abortion or neurologic disease. EHV-4 virus is considered as clinically & economically important viral pathogen which causes severe economic losses to horse industry worldwide. EHV-4 infection is enzootic in Indian domestic horse population. It is responsible for upper respiratory tract disease in horses of approx. all ages

characterized by short incubation period and symptoms include fever, coughing, profuse serous nasal discharge, lethargy, anorexia and variable enlargement of mandibular lymph nodes. Respiratory illness caused by Equine HerpesVirus type 4 badly affects the racing performance and make them susceptible to otherrespiratory pathogens like strangles. Most damage occur after secondary bacterial infections. Life-long latency and reactivation after primary infection are the major factors of epidemiology of EHV-4 infection. So, effective diagnosis, prevention & control of the disease is very important.

Diagnosis of EHV-4 infection can be done by different methods such as Virus Neutralization Test, CFT, PCR assays and sequencing. Since standard serological methods are time and labour intensive, PCR has been proved as a very rapid, effective & sensitive diagnostic test for EHV4infection.

EHV-1 and EHV-4 have highly cross reactive polyclonal antibodies which makes their differentiation difficult by using conventional diagnostic tests such as CFT, VNT and other polyclonal antibody based immunoassays. It is very important to have a test which can distinguish between the two infections and simultaneously help in sero-epidemiological studies to know the status of EHV-1 & 4 infections. So the inability of distinction between Equine Herpes Virus type 1 and 4 antibodies by VNT & CFT is overcome by the use of recombinant gG based Type-specific ELISA which detect Abs to immunodominat type specific epitopes of glycoprotein G of EHV-1 & 4 viruses. Testing of the serum samples using type-specific ELISA gave a very high positivity of the antibodies to EHV-4. The same results have been obtained inother parts of the world in various studies, the type-specific Enzyme-Linked Immunosorbent Assay has other important application in the management of disease outbreaks and has considerable potential in future EHV-1/4 vaccine development in allowing differentiation of vaccinated from diseased horses.

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