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Review Article
Equine Herpes Virus Type-1 Infection: Etiology, Epidemiology, Pathogenesis, Identification and Recent Diagnosis

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Abstract
To date, the family of herpesviruses comprises important human and many veterinary relevant pathogens encompassing more than 100 herpesviruses that infect vertebrates. A common feature of herpesviruses is their ability to infect the host for life. Equine herpesvirus type 1 (EHV-1) is one of the most important causes of respiratory diseases in horses and may cause abortion and nervous manifestations (equine herpes myeloencephalopathy) with frequently fatal outcome. The present review article was aimed to through light about the etiology, epidemiology, pathogenesis and diagnosis of Equine herpesvirus type 1 infection. Epidemiology of EHV-1 included the history of the disease, distribution and prevalence of EHV-1, routes 0f transmission, risk factors and latency. Enzyme Linked Immunosorbent Assay (ELISA) and Virus Neutralization Test (VNT) are highly recommended by the Office International des Epizooties (OIE) either for EHV confirmation of clinical cases or prevalence of infection surveillance. Isolation and identification of EHV-1 from nasal or nasopharyngeal swabs or buffy coat samples are strongly supportive of diagnosis of Equine Herpes Myeloencephalopathy (EHM) in a horse with compatible clinical signs and considered as the 'golden standard test' for a laboratory diagnosis of EHV-1 infection and should be attempted, especially during epidemics of EHM, concurrent with use of rapid diagnostic tests such as the Polymerase Chain Reaction (PCR), in order to achieve retrospective biological and molecular characterization of the viral isolate.

Key words: Equine, herpes virus, etiology, epidemiology, pathogenesis and diagnosis

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INTRODUCTION

This review is a novel article summarized the current state of Equine herpes virus type-1 infection: Etiology, epidemiology, pathogenesis, identification and recent diagnosis as a biological or molecular characterization of the viral causative agent of this infection.

Fortunately, horses have a limited number of diseases that can be transmitted to humans under natural circumstances. However, due to the close contact of horses as work or consumption of horse meat with many people in some countries, exhibition or companion animals, human exposures to horse diseases can be more numerous than for other large animals. Some equine zoonoses are significant and emerging, such as Equine herpesvirus Hendra virus, while others are well known: anthrax, salmonellosis Toxoplasmosis and rabies[1-3].

Equine herpesvirus type 1 (EHV-1) is known as ubiquitous pathogen in horse populations throughout the world and commonly associated with respiratory disease in young horses. However, it is also associated with late-gestation abortion, prenatal mortality and myeloencephalitis[4,5].

Equine herpesvirus 1 (EHV-1) is a member of the Alpha herpes virinae subfamily along with Varicella-Zoster Virus (VZV), pseudorabies virus (PRV), bovine herpesvirus 1 (BHV-1) and herpes simplex virus type 1 (HSV-1)[6].

EHV-1 is highly contagious and usually transmitted by direct contact mainly through the infected nasal discharge between infected animals or animal and an infected object. While rare, EHV-1 can be transmitted by aerosol or contaminated feed, water and equipment[5,7].

The outcome of EHV-1 infection is influenced by several factors including the age, the physical condition and the immune status of the host, the type of infection (primary, reinfection and reactivation) and the pathogenic potential of the virus strain[8,9]. Stress, fall and spring seasons and animals older than 5 years of age had a period of pyrexia. These risk factors were predictive of neuro-virulence and death[10]. Mares and foals populations were found to be a reservoir of EHV-1, from which the virus can be transmitted before and after weaning and as young as 30 days of age[11].

Peptide specific Enzyme Linked Immunosorbent Assay (ELISA) had been used to examine the presence of specific antibodies against equine herpesvirus type 1 (EHV-1) and equine herpesvirus type 4 (EHV-4) in a large horse population, 82% of the tested animals were positive for EHV-1 and 95% were positive for EHV-4, the high prevalence of serum neutralizing antibodies were expected due to the strong antigenic cross reactivity between the two viruses[12].

The objective of this review article was to illustrate some etiological, epidemiological, pathogenesis and diagnostic features of EHV-1 infection.

Etiology and molecular structure of equine herpes virus: Equine herpesvirus type 1 (EHV-1) is a member of the subfamily *Alphaherpesvirinae* and the genus *Varicellovirus*[5,13]. It is considered as one of the most important causes of respiratory disease in horses and can also causes abortion and nervous manifestations (equine herpes myeloencephalopathy) with multiple death occurrence[14]. The EHV-1 is classified as a member of the *Alphaherpesvirinae*, a subfamily of the *herpesviruses* that is typified by herpes simplex virus type 1 (HSV-1) and also includes Varicella-Zoster Virus (VZV) and pseudorabies virus (PRV)[9]. The functions of EHV-1 proteins are thought to be similar to those of herpes simplex virus type 1 (HSV-1)[15]. Asinine herpesvirus 3 (AHV-3) (also known as EHV-8) was first isolated from donkeys following high-dose corticosteroid administration[16]. Phylogenetic analysis has shown that AHV-3 is more closely related to EHV-1 than EHV-4 [17]. The latest taxonomy summarizes herpesviruses within a new order, *Herpesvirales*, which is divided into three families: *Herpesviridae, Alloherpesviridae* and *Malacoherpesviridae*. The family *Herpesviridae,* representing the mammals, birds and reptiles viruses, consists of three subfamilies namely the *Alpha*-, *Beta*- and *Gammaherpesvirinae*. In equids, nine herpesviruses have been identified so far, of which EHV-1 (equine abortion virus), EHV-3 (equine coital exanthema virus), EHV-4 (equine rhinopneumonitis virus), EHV-6 (asinine herpesvirus 1), EHV-8 (asinine herpesvirus 3) and EHV-9 (gazelle herpesvirus 1) belong to the genus *Varicellovirus* of the subfamily *Alphaherpesvirinae*. The other three viruses, EHV-2, EHV-5 and EHV-7 (asinine herpesvirus 2), belong to the *Gammaherpesvirina*e subfamily[13]. The horse is the natural host to EHV-1, -2, -3, -4 and -5, EHV-1 is considered to be the most important due to its diverse clinical presentations and the potential to cause high economic losses[18]. While the donkey is the host to EHV-6, -7 and -8 [2]. The EHV-9 is a neurotropic virus and was originally isolated from gazelle[19]. More recently, a new member of the *Gammaherpesvirinae* subfamily, zebra herpesvirus 1, was identified by Polymerase Chain Reaction (PCR) from blood samples collected from a mountain zebra in Namibia[20]. The herpesvirus virion consists of four morphologically distinctive components: The inner nucleoprotein core containing a linear double-stranded genomic Deoxyribonucleic acid (DNA), the icosahedral capsid, the tegument and the envelope[21], whereas the viral envelope proteins have been extensively studied, the proteins in the

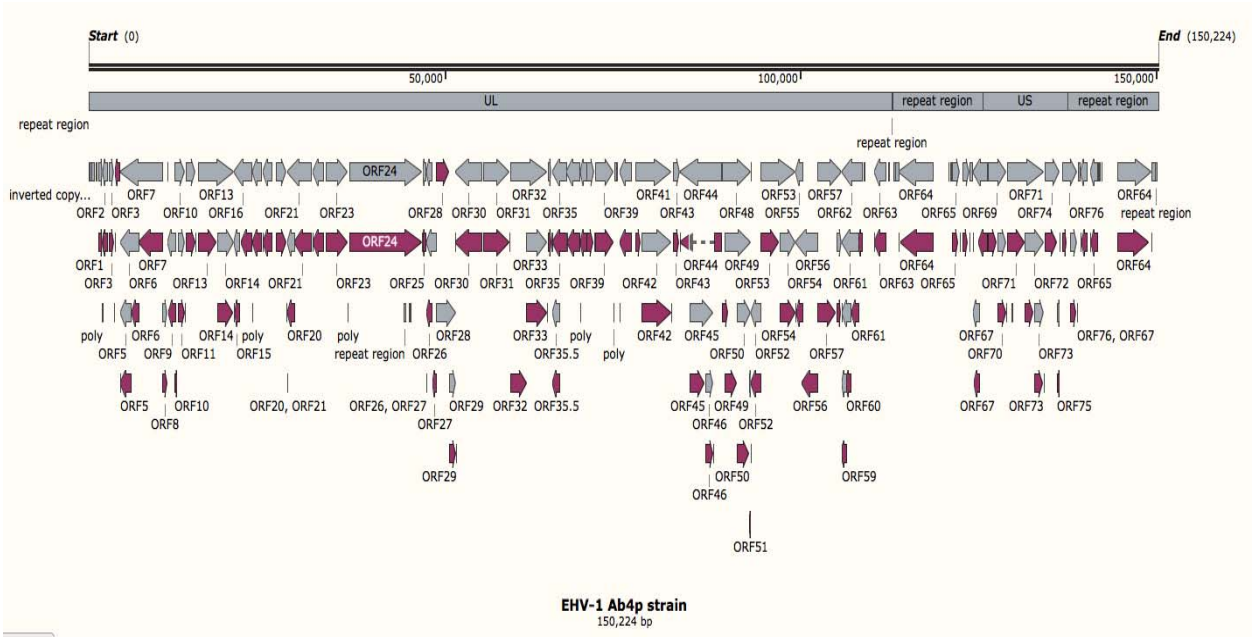

Fig. 1: EHV-1 Ab4p strain genomic map created using Snap Gene software program

tegument of EHV-1 are poorly characterized. Herpesviruses acquire their final tegument and envelope in the cytoplasm after passing through the nuclear membrane. This process includes acquisition of a primary envelope by budding of nucleocapsids at the inner leaflet of the nuclear membrane and subsequent fusion at the outer leaflet[22]. The complete DNA sequence of a pathogenic British isolate (strain Ab4) of EHV-1 was determined, it is a double-stranded DNA approximately 150 kbp in size separated into a Unique Long (UL) region and a Unique Short (US) segment, the latter being bracketed by two inverted repeat regions called the Internal Repeat (IR) and Terminal Repeat (TR)[15,23] as shown in Fig. 1, this arrangement is similar to the HSV-1, VZV and PRV genomes and contains 80 Open Reading Frames (ORFs), corresponding to 76 distinct genes, likely to encode proteins, as shown in Fig. 1. Comparison of predicted amino acid sequences to those of HSV-1, VZV and PRV homologs allowed the functions of many EHV-1 proteins[13,15]. Comparison of the sequences of EHV-1 and the human pathogens herpes simplex virus type 1 (HSV-1) and Varicella Zoster Virus (VZV) revealed that most of the genes are conserved between the viruses. Among the predicted genes in Ab4 strain of EHV-1, however Telford *et al.*[15] and many authors find six genes, ORFs l, 2, 3, 34, 59 and 67, which lack positional and/or sequence homologs in HSV-1. It is recorded that, EHV-1 gene expression is controlled by viral negative and positive regulatory molecules and is divided into Immediate-Early (IE), early and late stages[24-26].

Epidemiology of equine herpes virus-1

History of the Disease: EHV-1 was firstly described at Kentucky agriculture experimental station in the beginning of the 1930s [27], after that it was described as an outbreak caused by the same virus in Spain affecting nearly half of 125 mares[28]. The first isolation of equine abortion virus from aborted foeti and 2 foals died shortly after birth had been recorded in Germany[29]. Diagnosed cases of abortion and neonatal death caused by EHV-1 in New South Wales as well as old cases of Western Australia. EHV-1 is common in the United States but rarely occurred in the United Kingdom, Australia and New Zealand[30]. The initial identification of the virus was done in the United States. The relation between the virus abortion and the respiratory infection was firstly assumed by a European observation[31]. Recorded an outbreak of EHV-1 in 1991 at a riding school in Netherland. Since 1966, several Equine Horse Myeloencephalopathy (EHM)[32] and also, outbreaks have been described in veterinary literature from Europe, North America and recently from Australia[33]. There are no confirmed reports of EHM from Iceland, Africa, South America or Asia (with the exception of Japan)[34]. Concerning Egypt, EHV infections did not receive much concern in literature since the early detection of the EHV-2 antibodies in Egyptian horse sera during a serological survey at 1965 [35]. The virus was isolated from aborted fetus on the chorio-allantoic membrane of embryonated chicken eggs as a first record by Hassanain *et al.*[36]. This trial was followed by other

succeeding ones by some authors, who isolated and identified the local EHV-1 strain from aborted Arabian mares and internal organ of their foeti from a private stud with a history of recurrent abortion during 2005 and 2006 [37,38].

Distribution and prevalence of EHV-1: Sero-epidemiological investigation at a large Thoroughbred (TB) stud farm in the Hunter Valley of New South Wales showed that more than 99% of the tested mares and foals were EHV-4-antibody positive, while the prevalence of EHV-1 antibody positive mares and foals were 26.2 and 11.4%, respectively[39]. The EHV-1 seroprevalence among New Zealand TB increased with age from 29% among 6-12 months old, 48% among 13-24 months old, to approximately 70% among adult (>2 years old) horses[40]. Similarly, 56% of 21 TB yearlings and 67% of 45 horses from outbreaks of respiratory disease tested positive for EHV-1 antibody in another New Zealand based study[41]. Serological surveys from different countries have consistently shown that EHV-1 appears to have a significantly lower prevalence than EHV-4, which could be partially explained by the finding that EHV-4 infection can occur throughout the year but EHV-1 infection mainly occurs in the winter (pregnancy) season[5]. Investigating the prevalence of EHV-1 infection based on isolation of the virus from latently infected leukocytes by co-culture with permissive cell lines *in vitro* or by PCR- based detection of viral DNA in latently infected cells. The results of these studies varied, although all demonstrated that a considerable proportion of the tested horses were latently infected with EHV-1 [42]. Some of the recent studies differentiated between neuropathogenic Open Reading Frame (ORF) (ORF30 D752) and non-neuropathogenic (ORF30 N752) genotypes of EHV-1. The results of those studies indicated that the neuropathogenic genotype is present worldwide[43]. Despite the fact that the ORF30 D752 equine herpes-1 viruses were detected in samples collected as far back as 1950s [44], the reasons for the perceived increase in the incidence of outbreaks of neurological disease and the apparent increase in the prevalence of ORF30 D752 viruses, are not well understood. Some authors observed an apparent association between more frequent vaccination against EHV-1 and increased likelihood of development of EHM[1]. However, Lunn *et al.*[18], pointed out that such data should be interpreted with caution because of the presence of a number of factors as horses with a history of frequent vaccination against EHV-1 also tend to be older than horses without such history[10]. In addition, results of prevalence studies may be confounded by the fact that latently infected horses can carry both genotypes of the virus[42]. The increased numbers of reported cases of EHM may also simply reflect heightened awareness of EHM amongst veterinarians, coupled with the improvements in molecular-based diagnostic capability. The EHV-1 had been considered as a listed and reportable disease in Israel, between January and May 2006 a large-scale outbreak with 35 cases of EHV occurred as a result of this outbreak, routine vaccination was initiated 6 years later only 3 cases were diagnosed. In 2012, 16 cases were diagnosed in one farm, which resulted in two deaths[45]. According to the reports of the Office International des Epizooties (OIE), EHV infection was not reported in neighboring countries (Jordan, Lebanon, Egypt and Syria) at least since 2005. Seroprevalence of EHV-1 and 4 was conducted In Israel and the result for EHV-1 was very low (<1%) while for EHV-4 was very high (>99%), raising questions regarding the appropriate vaccination guidelines[46].

Routes of transmission: EHV-1 is highly contagious and usually transmitted by direct contact, mainly through the infected nasal discharge between infected animals or animal and an infected object. While rare, EHV-1 can be transmitted by aerosol or contaminated feed, water and equipment[2,4]. Gardiner *et al.*[47], revealed that fetal and placental tissues from EHV-1 abortions typically contain large quantities of infectious EHV-1 that comprise an excellent source of infection to other horses. This can occur through either direct contact with the infectious material (e.g., for paddock mates) or through fomites (e.g., shoes or clothing of grooms, handlers and veterinarians). It is still unclear whether the virus can actually spread by the venereal route or not. Previous reports studied the possible risk of horizontal transmission of EHV-1 via semen and their effect on stallion fertility. By using conventional PCR, EHV-1 DNA in 51 out of 390 total semen samples (13%) were detected, however the presence of EHV-1 did not appear to affect the fertility of infected stallions[48]. In another study, using real-time PCR, EHV-1 shedding could be detected in semen on day 20 after the onset of fever in naturally infected stallions, which seems not to be directly associated with spermatozoa[49]. One of the major challenges in controlling the spread of EHV-1 is recognizing which horses are likely to be shed the virus and therefore would represent a contagious disease risk. There is little information regarding the duration of nasal shedding of EHV-1 that can be expected in naturally infected horses with EHM. Despite the previously held belief that horses exhibiting signs of EHM were no longer shedding virus. Findings from a large, well-publicized outbreak clearly demonstrated the threat of nosocomial infection that can be associated with hospitalization and care of horses affected by EHM[50].

Risk factors: The outcome of EHV-1 infection is influenced by several factors including the age, the physical condition, the

immune status of the host, the type of infection (primary, reinfection and reactivation) and the pathogenic potential of the virus strain[9], Ata[51], also, the state of pregnancy and the different localities significantly affect the prevalence of EHV-1 infection. Stress, fall and spring seasons and animals older than 5 years of age had a period of pyrexia equal to or greater than 103.5°F and a peak temperature occurring on day 3 of the febrile period. These risk factors were predictive of neuro-virulence and death[10]. Mares and foals populations were found to be a reservoir of EHV-1, from which the virus can be transmitted before and after weaning and as young as 30 days of age[11]. Equine herpesvirus associated myeloencephalopathy could be developed during outbreaks of EHV-1 infection. The disease can affect up to 50% of the exposed horses. This percentage could be fluctuated significantly among horse populations and it strongly depends on the demographics and risk factors. The risk of EHM development was shown to be high in older horses[34], pregnant mares and mares with foals at foot[52] Older animals (>20 years old) were also at high risk for development of EHM following experimental infection with EHV-1; while, EHM was experimentally induced in three out of four mature mares[34]. While experimental infection of foals with a highly virulent strain of EHV-1 resulted in only mild respiratory disease[53]. Most reports of EHM outbreaks occurred more frequently among stabled horses than those at pasture, which may be related to conditions favoring transmission of any infectious agents due to presence of large numbers of clinically affected horses, higher traffic of both people and horses in and out of stable yards, using of common equipment that may act as fomites and stabled horses are also more likely to be used in training and competing and, thus, are presumably more stressed than those at pasture, which may facilitate reactivation of latent EHV-1 [54]. Herpes viruses are generally species-specific with limited reports of inter-species transmission but here is increasing evidence that the host range of EHV- 1 goes far beyond equine species[55]. It has been reported that non-equids, including llamas (*Lama glama*), alpacas (*Vicugnapacos*), blackbuck (Antelopacervicapra), including Persian onagers (*Equushemionusonager*), Damara zebra (*Equusquagga*), Grant's zebra (*Equusquaggaboehmi*), Burchell's zebra (*Equusquaggaburchelii*), Grevy's zebras (*Equusgrevyi*) and Thomson's gazelle (*Eudorcasthomsoni*) were susceptible for infection[56-58]. Recently, neurotropic EHV-1 strains were isolated from black bears (Ursus americanus), Thomson's gazelles and guinea pigs that suffered from severe neurological disorders[59]. A new recombinant EHV-1/EHV-9 virus had been identified. The virus seems to have originated in zebras but it was unclear whether the recombination occurred in captive zebras in zoos or wild zebras in natural habitats before importation. The recombination resulted in a pathogen which was fatal for polar bears, while zebras remain asymptomatic and likely carry the virus as the definitive host. It is still unclear whether EHV-1/EHV-9 recombination is a single event or happens more frequently[60]. A closely related but distinct virus, EHV-9, was first described in Thomson's gazelles (Eudorcasthomsoni) suffering from neurological symptoms[19]. Although it is suggested that equids are the natural and definitive host as many zebras were reported to be seropositive for EHV-9, it has also been suggested that animals other than horses may also play a role in the epidemiology of EHV-1 [59]. It has been proposed that donkeys and mules may act as silent carriers in the face of a neurological EHV-1 outbreak among horses and should be considered in outbreak management plans[42]. Recrudescence from latency will cause viral respiratory tract replication, which may then start further horizontal spread to other in-contact horses, this horizontal spread through respiratory droplet infection or fomites transmission but also contact with an aborted fetus or fetal fluids or membranes, is considered the second mechanism by which EHV-1 or -4 maintains presence in equine populations[18,61]. Foals have been shown to be infected within the first few months of life and shedding of EHV-1 has been detected in populations of brood-mares and their foals[39], often in the presence of maternally derived antibodies, with or without accompanying signs of respiratory disease[62]. Transmission of the exogenous virus between horses or reactivation of the latent virus it seems that both mechanisms contribute to the epidemiology of EHV-1 on farms[63]. Nasal shedding appears to be part of a "silent" cycle of herpesvirus infection. It is possible that the event of parturition with endo-crinological changes is linked to activation of latent herpesvirus in the mare followed with nasal shedding of virus. For this reason, postpartum mares should be considered an at-risk group for EHV-1 shedding. The ability of EHV-1 to establish infection in foals within their first few weeks of life constitutes an important epidemiological advantage for the virus; vaccination at such an early stage is unlikely to be successful due to the interference with maternally derived antibodies[62].

Latency: EHV-1 maintains the presence in equine populations via the latency, which is a non-replicative, non-immunogenic stage in a horse. The triggers for EHV-1 recrudescence, as well as the molecular mechanisms underlying this process, are poorly understood. The virus could be reactivated experimentally by administration of high doses of glucocorticosteroids[8]. Thus, in real life, stressful conditions

such as transport, sales, competitions have the potential to induce EHV-1 recrudescence[61]. The virus may also recrudesce in immunocompromised animals. The latter may provide an explanation for a silent circulation of EHV-1 among pregnant mares, as pregnancy has been shown to induce physiological immunosuppression in the horse[64]. Recrudescence of latent EHV-1 may[52] or may not be accompanied by clinical disease[8]. In either case, latently infected horses become infectious following EHV-1 recrudescence in the respiratory tract and hence comprise a source of EHV-1 to susceptible animals. Another important consequence of latency is the fact that sporadic cases of abortion and possibly neurological disease, can occur in a closed group of horses, without an external source of EHV-1 infection[65]. The fact that the majority of EHV-1 abortions occur as single events supports this view[66]. The source of the offending virus is thought to be EHV-1 that has reactivated locally within the blood vessels of the pregnant uterus and possibly, by extrapolation, the CNS. Such local reactivation can occur with or without the concurrent lytic respiratory infection and hence, with or without shedding of the virus in nasal secretions[61]. The initial respiratory infection that led to the establishment of latency could have happened at any time in the past, possibly months to years before EHV-1 abortion or neurological disease[7]. This provides an obvious challenge to the diagnosis and control of EHV-1-associated diseases. As with other herpes viruses, the ability of EHV-1 to infect horses and establish a long-term latent-carrier state in the face of the host immune responses assures indefinite endemic EHV-1 infection in the equine population. Resistance to re-infection resulting from recovery from field infection with EHV-1 is short-lived, lasting only a few weeks or few months. After infecting the horse via the respiratory tract, EHV-1 rapidly becomes intracellular, including within circulating lymphocytes and passes directly from cell to cell without an extracellular phase so that the virus is not exposed to neutralizing antibodies and other protective components of the immune response[67]. It is worth noting that the persistent (latent) infection site including Peripheral Blood Mononuclear Cells (PBMCs) and trigeminal ganglia, in order to be effective, EHV-1 vaccines must satisfy a challenging set of demands and activate a protective response that exceeds that provoked by natural infection[67].

Pathogenesis: Initially, EHV-1 infects epithelial cells of the nasal mucosa and/or nasopharynx[68]. Experimentally, the virus can enter the cell via a number of different pathways, either by direct fusion with the plasma membrane or by endocytosis followed by fusion with an endosomal membrane[69]. Equine major histocompatibility complex 1 (MHC-1) and cellular integrins have been identified as receptors used by EHV-1 [69,70]. It is likely that the virus can also use additional receptors, as yet unidentified, for entry into some cell types[71]. Following infection, the virus replicates in epithelial cells of the upper respiratory tract, resulting in virus shedding and distinct herpetic lesions of mucosal membranes. Spreading from cell to cell until reaching respiratory tract lymph nodes and the establishment of a lymphocyte associated viremia was conducted. This viremia is a prerequisite for abortion and neurological disease[5]. Different studies evaluated the role of EHV-1 genes in the pathogenesis at the cellular level including virus plaque formation, subcellular localization and spreading from cell to cell[3,51]. The local damage to the respiratory epithelium may predispose horses to infection with other respiratory pathogens. Indeed, detection of multiple respiratory pathogens from horses with clinical signs of upper respiratory disease is common[72]. After respiratory infection, EHV-1 invades the lamina propria using migrating mononuclear cells as vehicles for further distribution. As a result, EHV-1 can easily spread throughout the body via a cell-associated viremia, reaching the secondary sites of virus replication, mainly the vasculature of the pregnant uterus and/or the Central Nervous System (CNS)[73]. The virus induces abortion or neurological disease as a result of vasculitis, thrombosis and ischemic damage[74]. The ability to establish cell-associated viraemia is crucial to the pathogenicity of EHV-1 [43]. The highly virulent strains of EHV-1 seem to be able to establish cell-associated viremia of higher magnitude than those of lower virulence[75]. This feature is also thought to comprise one of the key differences between the pathogenic potential of EHV-1 and its close relative EHV-4 [73]. The latter replicates predominantly in the upper respiratory tract, produces low or undetectable cell-associated viremia and has rarely been implicated in outcomes other than respiratory disease of varying severity. During surveillance study that determined infectious agents in respiratory conditions in horses[42]. Mandibular lymphadenopathy and fever were the main clinical signs in EHV-1-positive horses. A fever can be expected during early upper respiratory tract replication of virus, which typically lasts from 36-48 h following nasopharyngeal instillation of a viral inoculum. A secondary fever is then noticed between days 5 and 10 following the infection and is due to a cell-associated viremia in peripheral blood mono-nuclear cells, it is a fact that high shedding during EHV-1 or 4 infection is associated with the febrile periods[34]. Using nasal mucosal explants as an infection model, it was shown that EHV-1 could spread from epithelium to the

connective tissue below the basement membrane, where mononuclear leukocytes were infected, whereas EHV-4-infected mononuclear leukocytes were extremely rare[75].

Diagnosis of EHV-1: The multifocal distribution of lesions caused by neurotropic EHV-1 strains results in considerable variability in clinical presentation and a number of conditions should be included in the differential diagnosis. These include equine protozoal myeloencephalitis, cervical stenotic myelopathy, cervical vertebral instability ('wobbler' syndrome), cervical vertebral fracture or other Central Nervous System (CNS) trauma, neuritis of the cauda equina, fibrocartilaginous infarction, aberrant parasite migration, degenerative myelopathy, togaviral encephalitis (flaviviruses and alpha-viruses), rabies, botulism, CNS abscessation and a variety of plant and chemical intoxications[76,77]. Samples might be collected from EHV-1 infection suspected cases are nasal swabs for detection/isolation of the virus, acute and convalescent coagulated blood (serum) for serology and anticoagulated whole blood for detection of cell-associated viremia. Age, gender, breed and EHV-1 vaccination history should be noted[43]. Neutralizing antibody titers to EHV-1 rises rapidly after natural infection and by 5-8 days after experimental infection[40]. Failure to demonstrate a 4 fold increase in antibody titer to EHV-1 in 10 of 19 neurologically affected horses was most likely due to delayed collection of acute samples, emphasizing the importance of obtaining initial samples as early as possible in the acute phase of infection. Because antibody titers are known to decline rapidly in infected or vaccinated animals, the elevated titers observed in the majority of exposed horses likely indicated a response to natural infection rather than residual vaccination titers. Previous reports have correlated the most severe neurologic signs with the most rapid increase in antibody titer after infection with EHV-1 [74]. Isolation and identification of EHV-1 from nasal or nasopharyngeal swabs or buffy coat samples are strongly supportive of diagnosis of EHM in a horse with compatible clinical signs and considered as the 'golden standard test' for a laboratory diagnosis of EHV-1 infection and should be attempted, especially during epidemics of EHM, concurrent with use of rapid diagnostic tests such as the PCR, in order to achieve retrospective biological and molecular characterization of the viral isolate, this requires submission of nasal or nasopharyngeal swabs in viral transport medium and an uncoagulated blood sample[67]. Results of virus isolation may, however, be negative because the peak of virus shedding has usually passed by the time neurological signs appear, virus may be shed intermittently and local antibodies may interfere with virus recovery[66]. The likelihood of isolating EHV-1 during outbreaks of neurological disease is increased by monitoring in-contact horses and collecting nasal or nasopharyngeal swab and buffy coat samples from these animals during the prodromal febrile phase before neurological signs develop[67]. Rapid laboratory tests for detection of EHV-1 are most useful in potential epidemics, because rapid identification of the causative agent is often critical for guiding management strategies. The PCR has become the diagnostic test of choice due to its high sensitivity and specificity. Detection of EHV-1 by PCR is routinely performed on secretions from nasal or nasopharyngeal swabs or from uncoagulated blood samples. Many conventional PCR detection protocols using single or nested methods and targeting specific EHV-1 genes have been published in recent years[78], since such assays are unable to differentiate between replicating (lytic), non-replicating or latent virus. Advances in technology and the use of novel PCR plat- forms, such as real-time PCR (RT-PCR), enable the quantification of viral loads for equine herpes viruses[79]. Serological surveys of EHV-1 and EHV-4 have always been complicated by the extensive antigenic cross reactivity, the virtual absence of type specific antibodies and widespread use of EHV-1 vaccination. The amount of sequence identity between EHV-1 and EHV-4, at the amino acid level, ranges from 55-96% across the genome[5,23]. This means that the two viruses cannot be distinguished antigenically using polyclonal antisera[80]. ELISA and Virus Neutralization Test (VNT) are highly recommended by OIE[81], either for EHV confirmation of clinical cases or prevalence of infection surveillance. Peptide-based ELISAs provide a simple, very specific, rapid, sensitive and relatively cheap diagnostic alternative and have been widely used for the serological diagnosis of multiple veterinary parasitic[82], bacterial[83] and viral infections[51,84]. Serology that demonstrates a 4 fold or greater increase in titer, using acute and convalescent samples, provides evidence of infection. Many horses with EHM, however, do not exhibit a 4 fold rise in Serum Neutralization (SN) titer, since titers rise rapidly and may have peaked by the time neurological signs appear[30]. Testing of sera from TB horses collected from 1967-1974 and 1993 gave values of 9 and 28% for EHV-1 and 100% for EHV-4 specific antibodies[85]. Hence the incidence of EHV-4 in adult TB horse population in Australia prior to the (1997) introduction of a killed EHV-1/4 vaccine was significantly higher than that of EHV-1. Similar results were reported by another Australian investigator, who found that 26.2% of 229 mare and 11.4% of their foals were positive for EHV-1 specific antibody on one stud farm in 1995 [11]. In studies conducted on large TB studs in

Australia using ELISA, serological evidence shows that EHV-1 infection was evident in 5/40 (12.5%) foals within the first 5 weeks of life[58] and in 10/26 (38.5%) foals aged 1-3 months[86]. Similarly, 3/27 (11%) foals on one American stud showed serological evidence of recent EHV-1 infection within the first month of life[87]. The prevalence of EHV-1 infection in equid species in Turkey using ELISA revealed that 14.5, 37.2 and 24.2% of the tested horses, mules and donkeys, respectively, were seropositive for EHV-1, while EHV-4- specific antibodies were detected in 81.7% of the tested horses. Both EHV-1 and EHV-4 DNA was detected from nasal swabs taken from symptomatic horses using multiplex PCR[88]. In a recent ELISA based serological study conducted in Egypt during 2016, it was cleared that the overall apparent prevalence rate was 64% (173/270), while, the true prevalence rate was 28% in average[51]. Hematological abnormalities in horses with EHM are inconsistent and may include mild anemia and lymphopenia in the early stages, followed a few days later by mild hyperfibrinogenemia[89]. Azotemia and hyperbilirubinemia occur secondary to dehydration and anorexia, respectively. Histopathological examination of the brain and spinal cord is essential in confirming of EHV-1 infection in a horse with suspected EHM. Vasculitis and thrombosis of small blood vessel[90] and virus detection in the CNS is achieved using immunohistochemistry, in situ hybridization and PCR[91].

CONCLUSION

Based on the data reviewed of this study, it was concluded that EHV-1 is considered as one of the most important causes of respiratory disease in horses and can also causes abortion and nervous manifestations with frequently fatal outcome. EHV-1 is highly contagious and usually transmitted by direct contact, mainly through infected nasal discharge between infected animals or animal and infected object. The risk of development of EHM was shown to be highest in older horses, pregnant mares and mares with foals at foot. ELISA and VNT are highly recommended to successfully examine the presence of specific antibodies against EHV-1 and EHV-4 in a large horse population. Many conventional PCR detection protocols using single or nested methods and targeting specific EHV-1 genes have been used in recent years, since such assays are unable to differentiate between replicating (lytic), non-replicating or latent virus. Advances in technology and the use of novel PCR plat- forms, such as real-time PCR (RT-PCR), enable the quantification of viral loads for equine herpes viruses.

SIGNIFICANT STATEMENT

Multiple risk factors including age, sex, state of pregnancy, latency and stress were found to play an important role in spreading of the disease within or between the equine herds. Accordingly, these factors should be taken in consideration in outbreak management, establishing of well-structured serological surveys especially those have the ability to differentiate between EHV-1 and EHV-4 are essential in control of the disease. ELISA is highly recommended for Equine herpes virus diagnosis, isolation and identification from nasal or nasopharyngeal swabs or buffy coat samples are strongly supportive of virus detection and as the different types of Polymerase Chain Reaction (PCR) and real time RT- PCR are rapid diagnostic tests in order to achieve retrospective biological and molecular characterization of the viral isolate and to differentiate between replicating (lytic), non-replicating or latent virus.

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