A review of equid herpesvirus 1 for the veterinary practitioner. Part B: Pathogenesis and epidemiology

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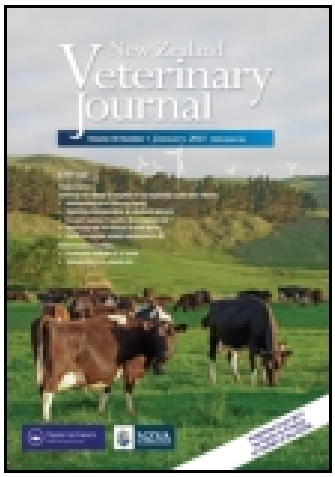

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A review of equid herpesvirus 1 for the veterinary practitioner. Part B: pathogenesis and epidemiology

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Review Article

A review of equid herpesvirus 1 for the veterinary practitioner. Part B: pathogenesis and epidemiology

M Dunowska*§

Abstract

Equid herpesvirus (EHV) type 1 is a common pathogen of horses with worldwide distribution. Infection with EHV-1 can be subclinical, or can result in sociologically and economically important outcomes such as abortion, neonatal death or neurological disease. The perceived recent increase in the reported cases of EHV-1 neurological disease in the United States of America and Europe over the past decade has caused concerns amongst veterinarians and horse owners worldwide. This review provides an update on the recent developments in our understanding of the pathogenesis and epidemiology of EHV-1 and associated diseases, with an emphasis on epidemiological data from Australasia. Many aspects of the pathogenesis and epidemiology of equine herpesvirus myeloencephalopathy still remain to be elucidated. This is an active area of current research worldwide.

KEY WORDS: *Equine herpesvirus 1, equid herpesvirus, EHV-1, equine herpesvirus myeloencephalopathy, EHM, neurological disease, EHV-1 epidemiology, EHV-1 pathogenesis, virus-host interactions*

Key points

- Many EHV-1 infections are subclinical, others are associated with respiratory disease of varying severity. Occasionally, EHV-1 infection can result in abortion, neurological disease or neonatal foal death.
- EHV-1 infection occurs through inhalation of the infectious virus. Horses shed EHV-1 in their respiratory secretions for up to 3 weeks post-infection. Other sources of infectious EHV-1 are fomites, and fetuses and placentas from EHV-1-induced abortions.
- Foals can become infected with EHV-1 early in life, often in the presence of maternally derived antibodies, which may or may not be accompanied by clinical signs of disease.
- EHV-1 isolates with different biological phenotypes exist, although the factors that influence the clinical outcome of EHV-1 infection in an individual animal are not yet fully understood.
- An apparent increase in numbers of reported outbreaks of EHV-1 neurological disease has been observed overseas over the past decade. The first confirmed outbreak of equine herpesvirus myeloencephalopathy (EHM) in New Zealand was reported in February 2014.
- Primary EHV-1 infection is followed by establishment of latency in neuronal or lymphatic tissues, from where the virus can periodically reactivate, which may or may not be accompanied by clinical signs of disease.
- The ability to establish cell-associated viraemia, with subsequent dissemination of virus throughout the body, is central to the pathogenicity of EHV-1.
- Infection of endothelial cells that line blood vessels in the gravid uterus and in the central nervous system results in severe vasculitis and multifocal thrombosis, which are thought to be responsible for abortion and neurological disease, respectively.

Introduction

Equid herpesvirus 1 (EHV-1) is a common pathogen of horses with worldwide distribution (Slater 2007). It is closely related to EHV-4, which was distinguished as a separate virus in 1981 (Studdert *et al.* 1981). Both EHV-1 and EHV-4 are large, enveloped, DNA viruses that are classified within the family *Alphaherpesviridae* in the order *Herpesvirales* (Davison *et al.* 2009). Infection with EHV-1 may be subclinical or accompanied by respiratory disease of various severity (Dunowska *et al.* 2002a). In addition, EHV-1 infection can also result in comparatively more serious clinical outcomes such as abortion (Carrigan *et al.* 1991), neonatal death (Frymus *et al.* 1986) or neurological disease (Friday *et al.* 2000).

The current review focuses on the recent data related to the pathogenesis and epidemiology of EHV-1-associated diseases, with the

CNS	Central nervous system
EHM	Equine herpesvirus myeloencephalopathy
EHV	Equid herpesvirus
MHC	Major histocompatibility complex
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
pfu	Plaque forming units
TB	Thoroughbred

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emphasis on epidemiological data from Australasia. The clinical presentation, diagnosis, treatment and prognosis for horses affected by EHV-1 disease are reviewed in a companion paper (Dunowska 2014).

Pathogenesis of EHV-1-associated diseases

The relationships between EHV-1 and its equine host are complex and not fully understood. It is currently unclear which viral and host factors are important for the clinical outcome of EHV-1 infection in an individual animal. For instance, despite close genetic and antigenic relatedness between EHV-1 and EHV-4, the former has the potential to cause more severe disease than the latter (Slater 2007). In addition, the existence of EHV-1 isolates with different biological phenotypes has been recognised by several authors (Smith *et al.* 2000; Tearle *et al.* 2003; Gardiner *et al.* 2012). The issue is especially puzzling considering the generally low level of genetic variability between different EHV-1 isolates (van Maanen *et al.* 2000). For example, there is only 0.1% difference between the sequences of EHV-1 strains with low (V592) and high (Ab4) virulence (Slater 2007). Which of those differences are associated with the markedly different biological properties of various EHV-1 isolates has not been fully elucidated, with the exception of dimorphism in the sequence of DNA polymerase, encoded by open reading frame (ORF) 30. A single substitution from aspargine (N) to aspartic acid (D), at amino acid position 752 of this gene, has been associated with increased neurovirulence (Nugent *et al.* 2006). However, not all EHV-1 isolates with N_{752} to D_{752} substitution induce neurological disease, and not all cases of EHV-1-induced neurological disease are caused by D_{752} viruses (Perkins *et al.* 2009; Cuxson *et al.* 2014). Therefore, it is likely that the viral markers of neurovirulence are more complex than this single amino-acid substitution (Pronost *et al.* 2010), and the importance of the D_{752} genotype should not be over-interpreted. It is recommended that similar disease control precautions are taken when infection with either genotype of the virus is identified (Kydd *et al.* 2012).

Equid herpesvirus 1 has been shown to enter cells *in vitro* by a number of different pathways, either by direct fusion with the plasma membrane or by endocytosis followed by fusion with an endosomal membrane (Azab *et al.* 2013). Equine major histocompatibility complex 1 (MHC-1) (Kurtz *et al.* 2010) and cellular integrins (Azab *et al.* 2013) have been identified as receptors used by EHV-1. It is likely that the virus can also use additional, as yet unidentified, receptors for entry into some cell types (Azab and Osterrieder 2012). It is currently unknown what factors determine the method of EHV-1 entry into the cell, and whether or not EHV-1 uses the same entry mechanisms *in vivo* as those described *in vitro*. Further knowledge of virus-receptor interactions is likely to facilitate development of future strategies to block such interactions and thus, to prevent the first step in EHV-1 infection.

Initially, EHV-1 infects epithelial cells of the nasal mucosa and/or nasopharynx, which results in epithelial cell damage (Gryspeerdt *et al.* 2010). 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 (McBrearty *et al.* 2013). The respiratory epithelium starts to recover from the virus-induced damage as soon as 3–5 days post-infection (Gryspeerdt *et al.* 2010). Unlike the situation observed with other alphaherpesviruses (Glorieux *et al.* 2011), the EHV-1-induced plaques do not appear to penetrate the basal membrane *in vivo* (Gryspeerdt *et al.* 2010) or *in vitro* during infection of nasal mucosal explants (Vandekerckhove *et al.* 2010). Despite the presence of the intact basal membrane, individual EHV-1-infected cells, comprising predominantly monocytes and T lymphocytes, can be observed in the connective and lymphoid tissues of the respiratory tract within 24–48 hours following experimental infection with the virus (Gryspeerdt *et al.* 2010). These *in vivo* observations were largely corroborated by the results of *in vitro* studies (Vandekerckhove *et al.* 2010). Hence, it appears that the ability of EHV-1 to infect cells of the immune system enables it to cross the basal membrane and disseminate to other organs within the body, including the pregnant uterus and the central nervous system (CNS). It also appears that the efficiency with which the virus can cross the basal membrane may be related to its virulence, as the numbers of EHV-1-infected cells below the basal membrane were higher in ponies infected with a neurovirulent strain of EHV-1 compared with a non-neurovirulent EHV-1 (Gryspeerdt *et al.* 2010).

The ability to establish cell-associated viraemia is crucial to the pathogenicity of EHV-1 (Kydd *et al.* 2012). The highly virulent strains of EHV-1 seem to be able to establish cell-associated viraemia of higher magnitude than those of lower virulence (Goodman *et al.* 2007). 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 (Osterrieder and Van de Walle 2010). The latter replicates predominantly in the upper respiratory tract, produces low or undetectable cell-associated viraemia, and has rarely been implicated in outcomes other than respiratory disease of varying severity.

The subpopulation of peripheral blood mononuclear cells (PBMC) responsible for the establishment of EHV-1 viraemia has not been consistent between results of a small number of *in vivo* studies (Smith *et al.* 1998; Gryspeerdt *et al.* 2010; Wilsterman *et al.* 2011). The issue is further complicated by the fact that *in vitro* susceptibility to EHV-1 infection seems to depend on the culture conditions. All PBMC fractions can be infected with EHV-1 following mitogen stimulation (van der Meulen *et al.* 2001), but in resting PBMC, infection is most efficient in monocytes (van Der Meulen *et al.* 2000). It is currently unclear what determines which subpopulations of circulating leukocytes become predominantly infected with EHV-1 *in vivo*, and whether or not these differ between EHV-1 isolates with different pathogenic potential. Although a small fraction of monocytes can become productively infected with EHV-1 *in vitro* (van Der Meulen *et al.* 2000), *in vivo* EHV-1 infection of leucocytes is believed to be either abortive or latent, without production of an infectious virus (van der Meulen *et al.* 2006). However, lytic EHV-1 infection can be established in other cell types that come in contact with EHV-1-infected leukocytes (Welch *et al.* 1992).

The EHV-1-infected leukocytes disseminate the virus to various organs (Figure 1). Infection of endothelial cells of blood vessels in the gravid uterus and CNS results in severe vasculitis and multifocal thrombosis, which are thought to be responsible for abortion (Smith and Borchers 2001) and neurological disease (Edington *et al.* 1986), respectively. The factors that are important for

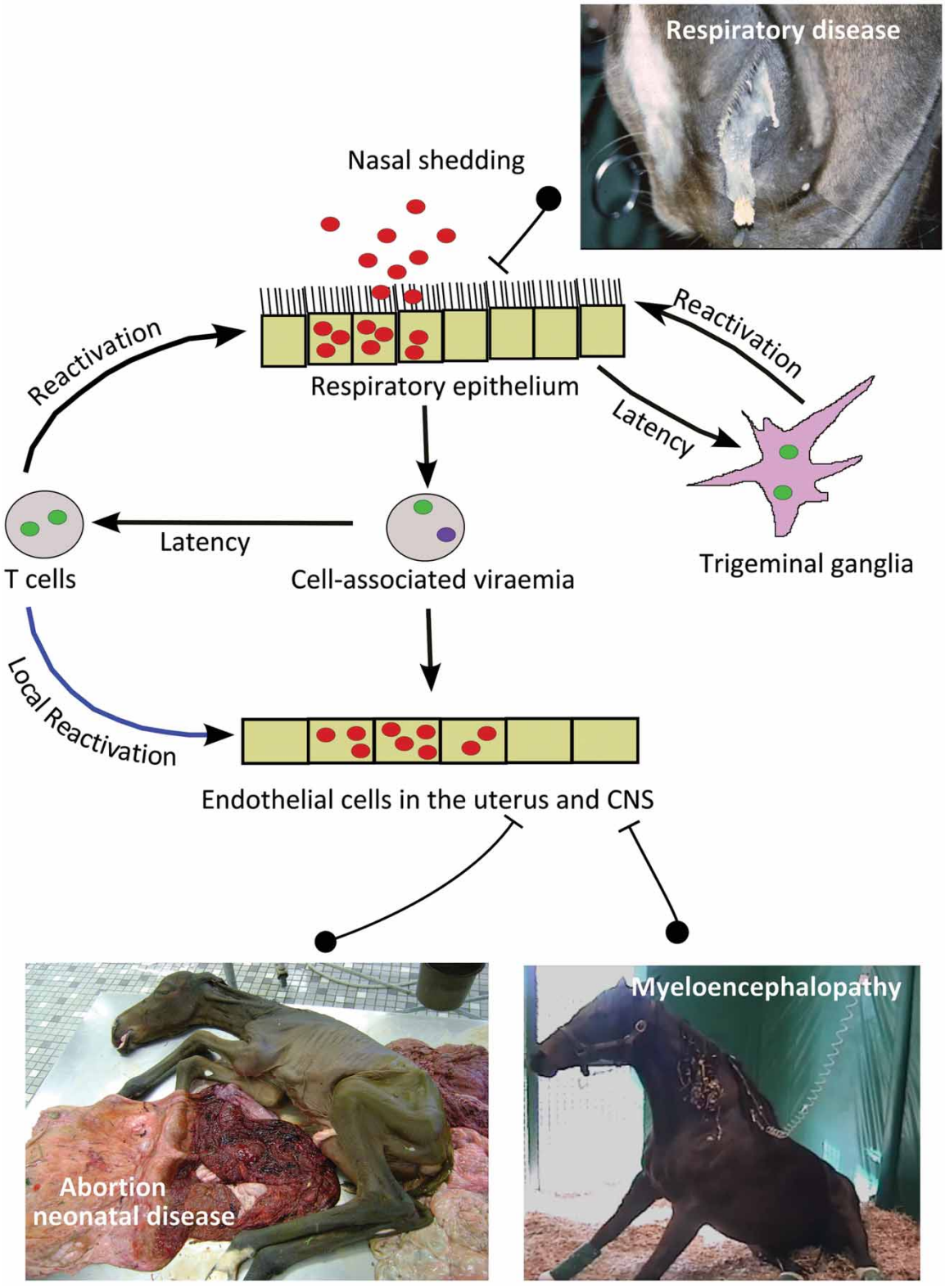

Figure 1. Schematic diagram of the proposed pathogenesis of equid herpesvirus 1 (EHV-1)-associated diseases. The initial lytic (red dot) infection occurs in the respiratory epithelium of the nasopharynx. Replication of EHV-1 in the respiratory tract may be asymptomatic or accompanied by clinical respiratory disease. The virus crosses the basal membrane in infected T lymphocytes and disseminates throughout the body via cell-associated viraemia. Translocation of EHV-1 to endothelial cells of the uterus and the central nervous system (CNS) may lead to abortion or development of equine herpesvirus myeloencephalopathy, respectively. EHV-1 infection around the time of parturition may result in birth of a weak, severely leukopenic foal. Infection of T lymphocytes is either abortive (purple dot) or latent (green dot). The EHV-1 latency can also be established in the trigeminal ganglia, probably by retrograde axonal transport of the virus following entry into sensory nerve endings in the nasal cavity. Recrudescence of the latent virus from either neural tissues or from T lymphocytes within the respiratory epithelium results in nasal shedding, cell-associated viraemia and the possibility of subsequent abortion or neurological disease. The virus may also reactivate locally within the endothelial cells of the uterus and the CNS, without the prerequisite for active infection of the respiratory epithelium (and nasal shedding of the virus) immediately prior to abortion or development of neurological disease. Photos courtesy of Joe Mayhew (respiratory disease), Rob MacKay (neurological disease) and Erica Gee (abortion).

initiation of endothelial cell infection from EHV-1-positive leukocytes are not well understood. The process may be facilitated by changes in cell surface molecules expressed by the endothelial cells of the gravid uterus in later stages of pregnancy (Smith *et al.* 2001). Other, as yet unidentified, mechanisms must play a role in translocation of EHV-1 to endothelial cells of the CNS. It is also possible that there are physiological triggers that act at the level of EHV-1-infected leukocytes to initiate the reactivation of the virus. Either way, the ability to infect endothelial cells comprises an important biological feature of the virus, as virulent EHV-1 isolates are more endotheliotropic than EHV-1 isolates of low virulence (Smith *et al.* 2000).

The molecular bases for differences in virulence between various EHV-1 isolates are not well understood. Several viral glycoproteins and tegument proteins have been investigated as potential markers of virulence due to their role in the early stages of infection (e.g. Thormann *et al.* 2012). It has also been suggested that the ability of the virus to modulate the host immune responses may affect its virulence. Some investigators demonstrated differences in chemokine expression observed in equine PBMS infected *in vitro* with EHV-1 strains of different pathogenic potential (Wimer *et al.* 2011). Those authors hypothesised that virulent EHV-1 may be able to induce increased local inflammation *in vivo*, which in turn may contribute to development of neurological disease, often termed equine herpesvirus myeloencephalopathy (EHM). Other viral characteristics that have been investigated include the ability of EHV-1 to down-regulate expression of MHC-1 proteins on the surface of infected cells (Rappocciolo *et al.* 2003; Ambagala *et al.* 2004). This feature has been linked to viral proteins UL56 (Ma *et al.* 2012) and UL49.5 (Koppers-Lalic *et al.* 2008). Interestingly, UL56 has been linked to the virulent phenotype of human herpes simplex virus 1 using experimental animal models (Kehm *et al.* 1996). By extrapolation, Soboll-Hussey *et al.* (2011) hypothesised that the decreased ability of EHV-1 strains without a functional UL56 (encoded by ORF1) to down-regulate expression of MHC-1 may facilitate the ability of the host to recognise and eliminate EHV-1-infected cells. However, although ponies experimentally infected with the EHV-1 ORF1/2 deletion mutant (and thus, without functional UL56) were febrile for a shorter time period and showed reduced nasal shedding of the virus, the overall clinical scores of all EHV-1-infected ponies were similar, irrespective of the genotype of the challenge virus (Soboll-Hussey *et al.* 2011). Altogether, these data indicated that while UL56 (ORF1) had some effects on clinical disease and immune responses, additional factors are likely to be important for the clinical outcome of EHV-1 infection.

Epidemiology and Transmission

Viral shedding and spread

Equid herpesvirus 1 infection occurs through inhalation of the infectious virus (Patel *et al.* 1982). Common sources of the virus include respiratory secretions of actively infected horses, fetuses or placentas from EHV-1 abortions, and fomites. The duration of shedding of EHV-1 from the respiratory tract is likely to be dependent on the immune status of the infected horse as well as on the properties of the virus. Experimentally infected animals can shed EHV-1 as early as Day 1 post challenge (Gardiner *et al.* 2012). Most horses cease shedding the virus within approximately 1–2 weeks post infection (Gibson *et al.* 1992b), although low levels of EHV-1 were detected in one case as late as Day 21 following experimental infection with EHV-1 (Perkins *et al.* 2008). Similarly, EHV-1 was intermittently detected in nasal secretions for between 6–17 days following corticosteroid-induced recrudescence of the latent virus (Pusterla *et al.* 2010a). Others reported a shorter duration (2 days) of nasal shedding following recrudescence (Gibson *et al.* 1992a). Overall, horses typically clear EHV-1 from their respiratory tract within 1–3 weeks post infection. Infected stallions can shed EHV-1 in semen, although the significance of this finding to the epidemiology of EHV-1 has not been determined (Tearle *et al.* 1996).

The load of EHV-1 in nasal secretions varies between horses, but the exact values reported are difficult to compare between studies due to differences in the sampling and laboratory methods employed. For example, maximum numbers of the live virus in 1 mL of medium used for collection of nasal mucous samples were approximately 10^3 plaque forming units (pfu) in one study (Hussey *et al.* 2006) and 10^5 pfu in another (Gibson *et al.* 1992a). In the latter study, samples were collected by gentle suction of nasal mucous, while in the former study via swabbing the nasal cavity. Using real-time PCR for assessment, the peak loads of viral DNA in nasal secretions of EHV-1-infected horses reached approximately 10 (Hussey *et al.* 2006) or 4.2×10^4 (Pusterla *et al.* 2009) viral gB copies per ng of template DNA. Another variable in the assessment of EHV-1 load in nasal secretions is the fact that the amount of EHV-1 shed differs throughout the period of shedding for each individual horse (Burgess *et al.* 2012). Hence, it would be expected to see high variability of EHV-1 load in horses sampled only once and at different stages of disease progression. Irrespective of the actual viral load, the virus is highly infectious, with infection rates of up to 100% in susceptible in-contact animals (Allen *et al.* 2004).

Fetal and placental tissues from EHV-1 abortions typically contain large quantities of infectious EHV-1 (Burrows 1970; Gardiner *et al.* 2012) and, therefore, 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).

Both EHV-1 and EHV-4 are environmentally labile and they are easily killed by treatment with detergents, lipid solvents, heat, and common disinfectants. Therefore, it is generally believed that EHV-1 does not survive well outside its equine host. However, experimentally, the virus has survived for up to a week at ambient temperatures when dried onto paper, wood or rope, and up to 35 days on horsehair or burlap (Doll *et al.* 1959). Hence, despite its fragile nature, EHV-1 can remain infectious on selected fomites under suitable conditions for up to a month, although the typical survival time would probably be much shorter.

Infections in foals

Foals can become infected with EHV-1 early in life, often in the presence of maternally derived antibodies, with or without accompanying signs of respiratory disease. In studies conducted on large Thoroughbred (TB) studs in Australia, serological evidence of EHV-1 infection was evident in 5/40 (12.5%) foals within the first 5 weeks of life (Foote *et al.* 2006) and in 10/26 (38.5%) foals aged 1–3 months (Gilkerson *et al.* 1997). Similarly, 3/27 (11%) foals on one American stud showed serological evidence of recent EHV-1 infection within the first month of life

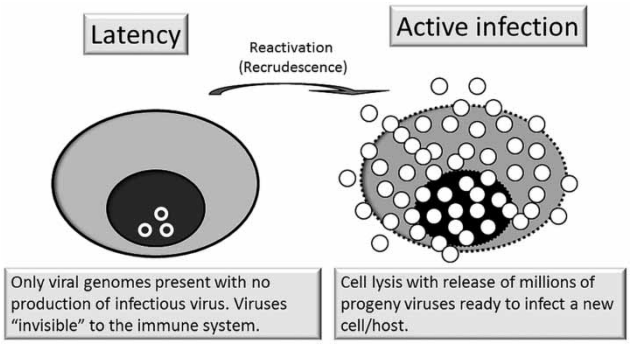

Figure 2. Equid herpesvirus type 1 (EHV-1) establishes latency in trigeminal ganglia and T lymphocytes. Infectious virus particles are not produced by latently infected cells. Following as yet unidentified triggers, EHV-1 can translocate to endothelial/epithelial cells in which it establishes lytic infection, with release of the infectious virus. Infection of T lymphocytes can also be abortive, in which case viral proteins are produced, but the production of mature infectious virus is aborted before completion

(Brown *et al.* 2007). In New Zealand, 18/23 (78%) TB foals followed on a monthly basis showed serological evidence of recent EHV-1 infection at about 4–8 months of age, and this was occasionally accompanied by respiratory disease (Dunowska *et al.* 2002a). Some of the foals also showed a second rise in EHV-1-specific antibody titres (determined by blocking ELISA) 5–6 months after primary infection, which was not accompanied by any clinical signs of disease. This second rise in the levels of EHV-1-specific antibody was observed only among foals from one of the three studs enrolled into the study.

Despite the serological evidence of EHV-1 circulation among foals, the frequency of EHV-1 detection was typically low in all studies that included a virological survey. Only six of 902 nasal swabs taken from 237 pairs of mares and foals in Australia over a period of 1 month tested positive for EHV-1 by PCR (Foote *et al.* 2004). Similarly, in a United States of America (USA)-based study, only four of the 1,330 samples (590 blood samples, 590 nasopharyngeal swab samples, 30 placentas, and 30 colostrum samples) collected from 75 horses and 30 foals over a period of 9 months tested positive for EHV-1 (Brown *et al.* 2007); and no EHV-1 positive samples were identified among 167 nasal swabs collected from 31 foals over a period of 8–13 months in New Zealand (Dunowska *et al.* 2002a).

Foals are believed to acquire EHV-1 infection from their dams, with potential for further foal-to-foal spread (Gilkerson *et al.* 1999b). Vaccination of mares with an inactivated EHV-1/4 vaccine did not seem to prevent EHV-1 circulation among populations of mares on Australian studs (Foote *et al.* 2006). It was not established whether the EHV-1 infections detected in the above two studies represented re-activation of the latent virus or transmission of the exogenous virus between horses. It is likely that both mechanisms contribute to the epidemiology of EHV-1 on stud farms (Gilkerson *et al.* 2000).

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. Furthermore, primary infection with EHV-1 results in the establishment of latency (Welch *et al.* 1992). Latently infected animals harbour EHV-1 in the episomal form in trigeminal ganglia (Slater *et al.* 1994) or in lymphoid cells (Chesters *et al.* 1997). By extrapolation from data for other alphaherpesviruses (Zaichick *et al.* 2011), the trigeminal neuronal latency is likely to be established by retrograde axonal transport of the virus following entry through sensory nerve endings in the nasal cavity. The latent virus does not produce any viral membrane glycoproteins, and infected cells are therefore not recognised by the immune system (van der Meulen *et al.* 2006). Latently infected horses do not shed EHV-1 and hence, are not infectious; they are also clinically normal (Allen 2006).

Viral latency and reactivation

Under appropriate conditions, the latent virus can undergo reactivation from latency, which is termed recrudescence (Figure 2). The triggers for EHV-1 recrudescence, as well as the molecular mechanisms underlying this process, are poorly understood. The virus can be reactivated experimentally by administration of high doses of glucocorticosteroids (Edington *et al.* 1985). Thus, in real life, stressful conditions such as transport, sales, competitions, or unsettled social structure have the potential to induce EHV-1 recrudescence. 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 (Noronha and Antczak 2012). Recrudescence of latent EHV-1 may (Gibson *et al.* 1992a) or may not (Edington *et al.* 1985) be accompanied by clinical disease. 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 (Figure 1).

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 (Crowhurst *et al.* 1981). The fact that the majority of EHV-1 abortions occur as single events supports this view (Mumford 1991). 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 (Slater 2007). 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 (Allen 2006). This provides an obvious challenge to the diagnosis and control of EHV-1-associated diseases.

Outbreaks of clinical EHV-1 disease

In addition to singular abortion cases, abortion storms, in which up to 50% or more of the expected foal crop may be lost, have been described worldwide (Carrigan *et al.* 1991). Such outbreaks have been very rarely documented in New Zealand (Donald 1998) or in Australia (Sabine *et al.* 1983; Carrigan *et al.* 1991). The first recognised EHV-1 abortion storm occurred in New Zealand in 1975, with three more occurring in 1977, 1988 and 1994 (Julian 1992; Donald 1998). The most recent case of multiple EHV-1 abortions was reported in 2010, following importation of a horse from Australia (Anonymous 2011a). The imported horse developed neurological disease and was subject to euthanasia. Subsequently, five pregnant mares at the stud aborted, including one that developed neurological disease.

While reports of EHV-1 abortion storms worldwide seem to have diminished in recent years, the numbers of reported outbreaks of neurological disease seem to have increased, causing concerns among veterinarians and horse owners (Kydd *et al.* 2012). As an example, one outbreak of EHV-1 infection with neurological presentations at a university riding centre in the USA involved 117 of 135 resident horses, 46 of which showed some degree of neurological signs. More than half of the latter were severely affected with grade 3 (out of 5) or above neurological deficits, with 14 fatal outcomes (Henninger *et al.* 2007). The economic impact of EHM on a horse industry can be illustrated by an outbreak of EHM at the National Cutting Horse Association Western National Championship event in Ogden, Utah in 2011 (Anonymous 2011b). The spread of EHV-1 at this event resulted in a total of 90 EHV-1 cases reported in 10 states, including 33 cases of EHM and 13 deaths.

Similar recent increases in the number of reported cases of EHM have not been recorded in New Zealand or in Australia. Only occasional cases of EHM have been reported in Australia (Studdert *et al.* 1984; Studdert *et al.* 2003; Cuxson *et al.* 2011). Recently, the first outbreak of EHM was reported in New Zealand (Anonymous 2014). Moreover, a case of a neurological disease associated with multiple EHV-1 abortions (Anonymous 2011a), as well as occasional detections of lesions suggestive of EHM aetiology in tissues submitted for pathological examination (Horner 1989) suggest that, similar to the Australian situation, sporadic EHM cases may have previously occurred in this country.

Prevalence of EHV-1 infections

The seroprevalence of specifically EHV-1 infections has been investigated in relatively few studies, due to the fact that most serological tests do not distinguish between EHV-1 and EHV-4 (Hartley *et al.* 2005). The serological testing is further complicated by a widespread use of EHV-1 vaccination. In Australia, prior to the introduction of EHV-1 vaccination in 1997 (Crabb and Studdert 1995), EHV-1-specific antibody was detected in 9% of randomly selected sera from 75 TB horses sampled between 1967 and 1974, and in sera from 28% of 97 racing TB horses in 1993. Similar numbers were reported by another Australian investigator, who found that 26.2% of 229 mares and 11.4% of their foals were positive for EHV-1-specific antibody on one stud farm in 1995 (Gilkerson *et al.* 1999a). Overall, 23% of 200 TB foals on two Australian stud farms were positive for EHV-1 antibody around and after weaning (Gilkerson *et al.* 1999b). By comparison, the seroprevalence of EHV-4 in the same studies was close to 100%, highlighting the differences in the epidemiology of these two viruses.

In contrast to the Australian studies, the EHV-1 seroprevalence among New Zealand Thoroughbreds 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 (Donald 1998). 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 (Dunowska *et al.* 2002b). Overall, these results suggest that EHV-1 infection is more common among New Zealand TB horses than among Australian TB horse populations. Alternatively, the differences between EHV-1 seroprevalence data from Australia and New Zealand may reflect the differences in the testing methods employed (van de Moer *et al.* 1993; Crabb *et al.* 1995).

Several authors investigated 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 horses tested were latently infected with EHV-1. In a British study, latent EHV-1 was detected in 50% of 40 horses at slaughter, often concurrently with EHV-4 (Edington *et al.* 1994). Pusterla *et al.* (2010b) detected latent EHV-1 in 26/147 (17.7%) horses, 2/4 (50%) mules and 2/2 (100%) donkeys at routine post-mortem examination at the University of California, Davis. In another study using the same methodology, latent EHV-1 was detected in 26% of 70 TB racehorses from four Californian racing tracks (Pusterla *et al.* 2012a). Finally, Allen *et al.* (2008) detected latent EHV-1 in 54% of 132 TB broodmares submitted for necropsy to the University of Kentucky. As with the serology data, it remains to be established whether discrepancies between the numbers reported reflect true differences between the sampled populations or simply the differences in the study design and testing methods used.

Some of the recent studies additionally differentiated between neuropathogenic (ORF30 D_{752}) and non-neuropathogenic (ORF30 N_{752}) genotypes of EHV-1. Results of those studies indicated that the neuropathogenic genotype is present worldwide (Kydd *et al.* 2012), including Australia (Cuxson *et al.* 2011), and New Zealand (unpublished data). Furthermore, the prevalence of the neuropathogenic genotype seems to have increased recently in the USA (Perkins *et al.* 2009), despite the fact that the ORF30 D_{752} EHV-1 viruses were detected in samples collected as far back as 1950s (Smith *et al.* 2010). The reasons for the perceived increase in the incidence of outbreaks of neurological disease, and the apparent increase in the prevalence of ORF30 D_{752} 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 (Reed and Toribio 2004). However, others pointed out that such data should be interpreted with caution because of the presence of a number of confounding factors (Lunn *et al.* 2009). For example, horses with a history of frequent vaccination against EHV-1 also tend to be older than horses without such history (Henninger *et al.* 2007). In addition, results of prevalence studies may be confounded by the fact that latently infected horses can carry both genotypes of the virus (Pusterla *et al.* 2012a). 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.

Risk factors for development of equine herpesvirus myeloencephalopathy

The risk factors for development of EHM are not fully understood. The reported outbreaks of EHM most often occurred among stabled, mature horses suggesting that age (adults *vs* foals) and management conditions (confined *vs* at pasture) may comprise risk factors for development of EHM (Kydd *et al.* 2012). In agreement with this view, the risk of development of EHM was shown to be highest in older horses (Goehring *et al.* 2006), pregnant mares, and mares with foals at foot (McCartan *et al.* 1995). Older animals (>20 years old) were also at increased risk for development of EHM following experimental infection with EHV-1 in one study (Allen 2008), and EHM was experimentally induced in three out of four mature female horses in

another study (Goehring *et al.* 2010). By comparison, experimental infection of foals with a highly virulent strain of EHV-1 resulted in only mild respiratory disease (Gibson *et al.* 1992a).

However, in most reports of EHM outbreaks, all horses were managed under similar conditions, making it difficult to assess the relative influence of stabling on the outcome of EHV-1 infection. The more frequent reports of EHM among stabled horses (e.g. van Maanen *et al.* 2001) than those at pasture (e.g. Barbic *et al.* 2012) may be related to conditions favouring transmission of any infectious agents, with a consequence of a larger numbers of clinically affected horses. These include higher traffic of both people and horses in and out of stable yards, and the use of common equipment that may act as fomites. Stabled horses are also more likely to be training and competing and, thus, are presumably more stressed than those at pasture, which may facilitate reactivation of latent EHV-1.

The importance of other factors on the outcome of EHV-1 infection remains to be established. Goehring *et al.* (2006) identified sex (females) and season (winter-spring) as factors associated with increased risks for EHM based on the analysis of six outbreaks of EHM in the Netherlands. Some authors suggested the existence of breed predisposition for the development of EHM. For example, Barbic *et al.* (2012) described predominantly neurological disease among Quarter horses on one stud farm, and predominantly abortions among Lipizzaner horses on another stud, during two epidemiologically linked outbreaks caused by genetically similar EHV-1. However, other researchers have not confirmed these observations (Allen 2008).

It has also been suggested that animals other than horses may also play a role in the epidemiology of EHV-1. Pusterla *et al.* (2012b) 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. In addition, EHV-1, or closely related virus, was recovered from cases of abortion in zebras and onagers (Montali *et al.* 1985), as well as from cases of perinatal mortality and neurological disease in zebras (Wolff *et al.* 1986). An EHV-1-like virus has also been occasionally recovered from diseased domestic and wild ruminants (Chowdhury *et al.* 1988) or camelids (Rebhun *et al.* 1988). The implications of these findings for EHV-1 epidemiology are currently unclear.

Altogether, the inconsistencies in both virological and serological data indicate that further research is needed in order to better understand the epidemiology of EHV-1 in different equine populations. The differences may exist between different geographical locations, management practices, as well as different breeds. The currently available data are heavily biased towards TB horses, with underrepresentation of other breeds.

Concluding remarks

Infection with EHV-1 is common among horse populations worldwide, including New Zealand. Despite the evidence that EHV-1 has been circulating among New Zealand horses and foals for close to 40 years or longer, herpesvirus-induced abortion storms have rarely been reported in this country, and the first confirmed outbreak of EHM occurred in February 2014. It remains to be seen whether the first New Zealand outbreak of EHM will remain an isolated incident, or if it will be followed by an increase in numbers of reported EHM cases, as has been observed in Europe and in the USA. While it's possible that viruses circulating in New Zealand are of lesser virulence than those present overseas, frequent movement of breeding and performance (e.g. racing) horses to and from New Zealand, combined with the reasonably high frequency of detection of latent EHV-1 overseas, makes it unlikely that New Zealand EHV-1 viruses are considerably different from those present overseas. Many questions regarding pathogenesis of EHV-1 infection and triggers for development of EHM still remain unanswered. Hence, in addition to the biological properties of the viruses circulating in the field, a number of host and environmental factors specific for New Zealand (e.g. pasture-based management and lack of a number of other equine pathogens that are present overseas) may also influence the probability of development of EHM following EHV-1 infection or recrudescence.

Equine herpesvirus abortion and neurological disease are not notifiable in New Zealand. It is likely that individual cases of EHM occurred in New Zealand prior to the outbreak in 2014, but were not diagnosed due to financial restrictions of the owner, inherent difficulties associated with EHM diagnosis, or failure of the attending veterinarian to consider EHM as a differential diagnosis for a horse with a sudden onset of neurological deficits. Both diseases have recently become notifiable in Australia (Anonymous 2013), which may lead to improved reporting and diagnosis of these economically important manifestations of EHV-1. This review aims to raise the awareness and understanding of the neurological presentations of EHV-1 infection among New Zealand veterinarians, so that suspect cases of EHM are recognised and investigated. Availability of the New Zealand-specific data would provide an opportunity to formulate evidence-based disease prevention and management plans based on the knowledge of local, as opposed to global, epidemiology of EHV-1-associated diseases.

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