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The increased prevalence of neuropathogenic strains of EHV-1 in equine abortions

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ABSTRACT

A panel of 426 archived EHV-1 isolates collected (1951–2006) from equine abortions was analyzed using a real-time Taq-Man® allelic discrimination PCR assay. Based on previous findings, isolates possessing adenine at nucleotide position 2254 (A_{2254}) in ORF30 were classified as having a non-neuropathogenic genotype and those with guanine at 2254 (G_{2254}) were designated as the neuropathogenic genotype. The resultant data demonstrated that viruses with the neuropathogenic genotype existed in the 1950s and isolates with this genotype increased from 3.3% in the 1960s to 14.4% in the 1990s. The incidence of EHV-1 isolates from 2000 to 2006 with G at position 2254 is 19.4%, suggesting that viruses with the neuropathogenic genotype are continuing to increase in prevalence within the latent reservoir of the virus, leading to greater risks for costly outbreaks of equine herpesvirus neurologic disease. Another highly significant finding was two isolates failed to react with either probe in the allelic discrimination assay. These isolates were found to possess an adenine to cytosine substitution at position 2258 ($A_{2258} \rightarrow C_{2258}$) in ORF30, in addition to $A_{2254} \rightarrow G_{2254}$. Interestingly, the non-neuropathogenic RAC-H modified live vaccine strain of EHV-1 also contains both $A_{2254} \rightarrow G_{2254}$ and $A_{2258} \rightarrow C_{2258}$ substitutions. This finding clearly suggests that additional research is required before the genetic basis of the neuropathogenic phenotype in EHV-1 is fully understood.

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1. Introduction

Equine herpesvirus-1 (EHV-1) infections cause significant economic losses for equine industries worldwide as a result of abortion, respiratory illness, and neurologic disease in all breeds of horses. Although it is believed almost all strains of EHV-1 can induce abortion in pregnant mares, only certain strains have the potential to cause neurologic disease (Nugent et al., 2006). Equine disease monitoring in Central Kentucky over the past 51 years (1957–2008) has confirmed the frequency of EHV-1 induced abortions has declined, even though the number of broodmares has increased threefold (Powell, 2008). The majority of such abortions in recent years have been single, sporadic events on individual farms among populations of mares that are routinely vaccinated against disease. In contrast, cases of EHV-1 induced neurologic disease have increased significantly in number since the year 2000 (Allen et al., 2008; Marenzoni et al., 2008; Patel and Heldens, 2005).[2] Within the United States and the United Kingdom, the number of reported outbreaks has risen from

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one occurrence in the early 1970s to 32 during the years 2001–2005 (Anonymous, 2007).[2] The associated case-fatality rate may also be increasing within the United States, ranging from 20% in some instances, to as high as 50% in others (Slater et al., 2006). Additionally, in 2005 significant outbreaks occurred in Canada, South Africa, Switzerland, Ireland and other European nations (Goehring et al., 2006; Slater et al., 2006).

EHV-1 is a member of the subfamily *Alphaherpesvirinae* with a 150 kbp double-stranded DNA genome, consisting of 80 open reading frames (ORFs), 76 of which are unique (Burton et al., 2001; Patel and Heldens, 2005). Within open reading frame 30 (ORF30), which encodes the viral DNA polymerase, a single nucleotide substitution is strongly associated with the occurrence of EHV-1 neurologic disease (Nugent et al., 2006). The exchange of adenine for guanine at position 2254 (ORF30; $A_{2254} \rightarrow G_{2254}$) results in an asparagine (N) to aspartic acid (D) substitution at amino acid position 752 ($N_{752} \rightarrow D_{752}$) (Nugent et al., 2006). This genotype was identified as the causative agent for 30 out of 32 investigated outbreaks of EHV-1 neurologic disease occurring in the United Kingdom and the United States, between the years 2001 and 2006 (Allen, 2007). The ability of EHV-1 strains possessing G_{2254} to induce neurological signs has also been proven through experimental infection of the horse (Goodman et al., 2007). Neuropathogenic EHV-1 strains are also able to replicate more efficiently and achieve 10-fold higher levels of leukocyte-associated viremia than observed in horses infected with non-neuropathogenic strains of EHV-1 (Allen, 2006a; Goodman et al., 2007; Van de Walle et al., 2009). The identification of this unique single nucleotide polymorphism (SNP) in ORF30 allowed for the development of a real-time PCR assay to discriminate between non-neuropathogenic and neuropathogenic strains of the virus (real-time Taq-Man® allelic discrimination PCR) (Allen, 2007; Allen et al., 2008).

EHV-1 establishes a life-long latent infection in a high percentage of animals following exposure to the virus (Allen et al., 2004; Allen, 2006b). Reactivation of the latent virus results in virus shedding for a limited period of time and the opportunity for transmission of the pathogen to susceptible, in-contact horses. Outbreaks of neurologic disease are thought to be initiated by viral reactivation and nasal shedding of neuropathogenic strains of EHV-1 by latently infected carriers (Allen and Timoney, 2007). The latently infected host is typically asymptomatic, although mares which harbor EHV-1 can abort their foals (Allen et al., 2004). While the primary site of latency is the lymph nodes associated with the respiratory tract, latent virus has also been detected in circulating lymphocytes and the sensory nerve-cell bodies of the trigeminal ganglia (Chesters et al., 1997; Edington et al., 1994; Slater et al., 1994). Where sporadic cases of abortion have been studied, the genotype of the strain of EHV-1 isolated from the aborted fetal tissues was found to be identical to the latent virus present in the mare that aborted (Allen et al., 2004; Allen, 2006b). In light of this observation, it was felt that archived EHV-1 isolates, derived from fetal tissues and recovered from sporadic equine abortions, would be an excellent source of material to study the distribution of both neuropathogenic and non-neuropathogenic strains of EHV-1 over an extended period of time, within the latent population of the virus. To date, no previously recorded studies have focused on identification of neuropathogenic strains of EHV-1 from sporadic cases of equine abortion. We hypothesize that the neuropathogenic strain of EHV-1 was associated with sporadic cases of equine herpesvirus abortion prior to the 1960s, and the proportion of EHV-1 abortion isolates which possess the neuropathogenic genotype has increased in recent years in comparison to previous decades in Central Kentucky. To test this hypothesis, a real-time Taq-Man® allelic discrimination PCR assay was used to analyze archived tissue culture fluid containing EHV-1 isolates recovered from tissues of foals aborted by Central Kentucky's Thoroughbred broodmare population during the past 46 foaling seasons, from 1950 through 2006.

2. Materials and methods

2.1. Cells

Confluent monolayers of fetal equine dermis (KyED) cells were maintained in 850-cm^2 tissue culture roller bottles in Eagle's Minimal Essential Medium (EMEM; Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (10% FBS) and gentamicin reagent solution (50-μg/ml; Invitrogen) as previously described (Allen et al., 1977; Allen and Turtinen, 1982; Turtinen et al., 1981). In the present study, the KyED cells were used between passages 7 and 10.

2.2. Viral isolates and generation of virus stock

Archived EHV-1 field isolates from Central Kentucky (Bourbon, Clark, Fayette, Franklin, Jessamine, Madison, Scott and Woodford counties), stored either as lyophilized or wet frozen (−70 °C) tissue culture fluid (TCF) stocks, were available for analysis. To ascertain if there was a time-related genetic shift in the latent EHV-1 reservoir, it was considered necessary to examine EHV-1 isolates from different decades, starting with the year 1950. Only isolates derived from fetal tissues of sporadic cases of abortion from Thoroughbred broodmares were included in this study. According to their case histories, none of the mares exhibited any signs of respiratory or neurologic disease prior to aborting. The number of EHV-1 isolates that met these criteria for each decade were as follows: 5 for the 1950s, 90 for the 1960s, 90 for the 1970s, 120 for the 1980s, 90 for the 1990s, and 31 for the 2000s ($n = 426$). In addition to these isolates, TCF containing 14 confirmed EHV-1 isolates from known equine herpesvirus outbreaks were included in the study: 7 neuropathogenic herpesvirus strains (G_{2254}; T313, T954, T955, T956, T964, T967, and T970) and 7 non-neuropathogenic herpesvirus strains (A_{2254}; T61, T75, T220, T493, T547, T572, and T812). The genetic identity of these 14 isolates was previously determined by sequencing ORF30. Additional TCFs, containing equine herpesviruses 1–5 were used as positive controls in the PCR amplification reactions. The EHV-1, 3, and 4 controls were obtained from the American Type