Short Communications

Investigation of the role of lesser characterised respiratory viruses associated with upper respiratory tract infections in horses

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VIRAL respiratory infections are one of the most common health problem in horses throughout the world (Traub-Dargatz and others 1991). Equine herpesvirus-1 (EHV-1), equine herpesvirus-4 (EHV-4) and equine influenza virus (EIV) are among the most common viruses associated with infectious upper respiratory tract disease (IURD) (Mumford and others 1998, Morley and others 2000, Mumford and others 2003, Pusterla and others 2005, Yactor and others 2006). These infections are often self-limiting and not life threatening, except in extreme cases. However, athletic horses generally have an increased risk of becoming infected, and disease associated with these infections has greater consequences for both the horse and owner/trainer because of the effect of disease on athletic capacity and ability to compete successfully (Mumford and Rossdale 1980). Horses with IURD generally develop similar clinical signs regardless of which primary or secondary agents are causing clinical disease. Disease is most frequently typified by the occurrence of fever, mucopurulent nasal discharge and coughing (Morley 1995, Dynon and others 2007, Diaz-Mendez and others 2010, Pusterla and others 2011). Despite intensive investigative efforts, veterinarians frequently diagnose clinical IURD without identifying a primary etiologic agent. Potential pathogens were not identified in 25–74 per cent equids with suspected IURD (Powell and others 1978, Burrows and others 1982, Ostlund and others 1991, Dynon and others 2007, Diaz-Mendez and others 2010, Pusterla and others 2011). The lack of aetiological diagnosis for some IURD cases observed in these investigations is at least partially attributable to concentrating diagnostic efforts on identifying infection with agents that most frequently cause disease. It is likely that more comprehensive diagnostic efforts would identify agents in affected animals that tend to cause either less dramatic outbreaks or sporadic rather than epidemic disease (eg, γ herpesviruses, equine rhinitis viruses, equine adenoviruses).

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The aim of this study was to investigate the frequency of lesser characterised respiratory viruses (equine herpesvirus-2 (EHV-2) and equine herpesvirus-5 (EHV-5), equine rhinitis A (ERAV) and B (ERBV), equine adenovirus 1 (EAdV1)) in nasal secretions of horses with confirmed causes of IURD (EHV-1/-4, EIV, *Streptococcus equi* subspecies *equi*), horses with signs of IURD, but no detected common respiratory pathogens, and healthy control horses.

Horses with IURD were selected from the diagnostic submissions to the Real-time PCR Research and Diagnostics Core Facility at the University of California, School of Veterinary Medicine, University of California at Davis from January 2009 to December 2011. Case selection included horses with either EHV-1 (40), EHV-4 (40), EIV (40) or *S equi* subspecies *equi* (44) infection confirmed by real-time PCR testing. Only horses with reported clinical signs of fever and nasal discharge were retained as study cases. An additional 172 horses with fever and nasal discharge, but negative results for EHV-1, EHV-4, EIV and *S equi* subspecies *equi* by real-time PCR served as IURD group. Emphasis was given to cases where an outbreak situation was likely to have occurred. Further, 39 healthy horses for export purpose, screened for respiratory pathogens, were included in this study as healthy controls.

For each selected case study, nucleic acid (genomic and complementary DNA) was available and stored at –20°C until used. Nucleic acid from nasal secretions was assayed for the presence of EHV-2, EHV-5, EAdV1, ERAV and ERBV using previously reported real-time TaqMan PCR assays (Bell and others 2006a, b, Mori and others 2009). Positive and negative controls were used for every PCR assay, and nucleic acid quality was assessed by analysing all extracted nasal secretions for the presence of the housekeeping gene equine glyceraldehyde-3-phosphate dehydrogenase (eGAPDH). Absolute quantitation of EHV-2 and EHV-5 target molecules was performed using standard curves for EHV-2/-5 and eGAPDH and expressed as EHV-2 or EHV-5 *gB* gene copies per million cells (Pusterla and others 2009).

Descriptive analyses were performed to evaluate the information from the submission forms. A Pearson's χ^2 test was performed to compare the frequency of the different viral pathogens among the horse groups. EHV-2 and EHV-5 viral loads for the different infectious disease groups were compared using the Wilcoxon-Mann-Whitney tests. For all statistical analyses, values of $P \leq 0.05$ were considered significant.

Age was available for the majority of the horses (30 EHV-1, 38 EHV-4, 40 EIV, 41 *S equi* subspecies *equi*, 161 IURD group horses, 39 healthy control horses), while breed and gender were generally not listed on the submission forms. The age range for each group of horses was similar and ranged from 2 months to 30 years. However, median age of the different horse groups varied with the lowest median age of one year reported in EHV-4 group horses, followed by EIV group horses (four years), IURD and healthy horse group horses (seven years), *S equi* subspecies *equi* group horses (eight years) and EHV-1 group horses (8.5 years). Frequency distributions of lesser respiratory viruses are reported in Table 1. The most commonly detected virus was EHV-5, followed by EHV-2, EAdV1 and ERBV. ERAV was not detected in the nasal secretions of any of the study horses. Statistical difference in EHV-2 distributions was determined between EHV-4 and EIV group horses ($P = 0.04$) and between, the EHV-4 group, the *S equi* subspecies *equi* group and the IURD group ($P = 0.01$). Further, statistical difference in EHV-5 and EAdV1 distributions were determined between EHV-4 and health control group horses ($P = 0.03$) and between *S equi* subspecies *equi* and IURD group horses ($P = 0.01$), respectively. Similar frequencies of EHV-2 alone (10–14 per cent), EHV-5 alone (18–21 per cent) or combined EHV-2/-5 (34–48 per cent) detection was recorded in the different horse groups. ERBV was associated with concurrent EHV-2 and EHV-5 detection in 7 horses, and with no EHV-2 and EHV-5 detection in an additional two horses. Six horses positive for EAdV1 also tested positive for EHV-2 and EHV-5, while EAdV1 was the only lesser characterised respiratory virus in an

TABLE 1: Frequency distribution of lesser characterised respiratory viruses in respiratory secretions of horses with confirmed causes of IURD, horses with signs of IURD but no detected common respiratory pathogens and healthy horses

Group (n)	Lesser characterised respiratory viruses				
	EHV-2	EHV-5	EAdV1	ERAV	ERBV
EHV-1 (40)	22	27	0	0	0
EHV-4 (40)	31	36	2	0	2
EIV (40)	14	23	1	0	1
Streptococcus equi subspecies *equi* (44)	27	27	4	0	2
IURD (172)	84	96	3	0	4
Healthy control (39)	7	16	0	0	0
Total (375)	185	225	10	0	9

EAdV, Equine adenovirus; EHV, Equine herpesvirus; ERAV, Equine rhinitis A virus, ERBV, Equine rhinitis B virus; IURD, Infectious upper respiratory tract disease.

additional four horses. EHV-2 viral loads for each group are reported in Fig 1. Significantly different EHV-2 viral loads were determined between *S equi* subspecies *equi* and IURD group horses (P = 0.01), and between healthy control horses and EHV-4, EIV, *S equi* subspecies *equi* and IURD group horses (P = 0.01, Fig 1). EHV-5 viral loads were significantly different between EHV-4 and the additional five groups (P < 0.0001, Fig 2).

The relative neglect in the investigation of lesser characterised infectious respiratory pathogens may be related to the dominant position of EIV and EHV-1/-4 as causes of acute upper respiratory diseases in horses, but is also related to a lack of sensitive, widely available and adopted diagnostic tests for lesser characterised respiratory viral pathogens. The past two decades have seen almost no discovery and characterisation of new equine respiratory pathogens, with the exception of Hendra virus, compared with the human medical field. The investigation of lesser characterised viruses and their possible interaction in coinfection is essential in order to improve the wellbeing of horses. It is imperative to gain a better epidemiological understanding of gamma herpesviruses, equine rhinitis viruses and adenoviruses,

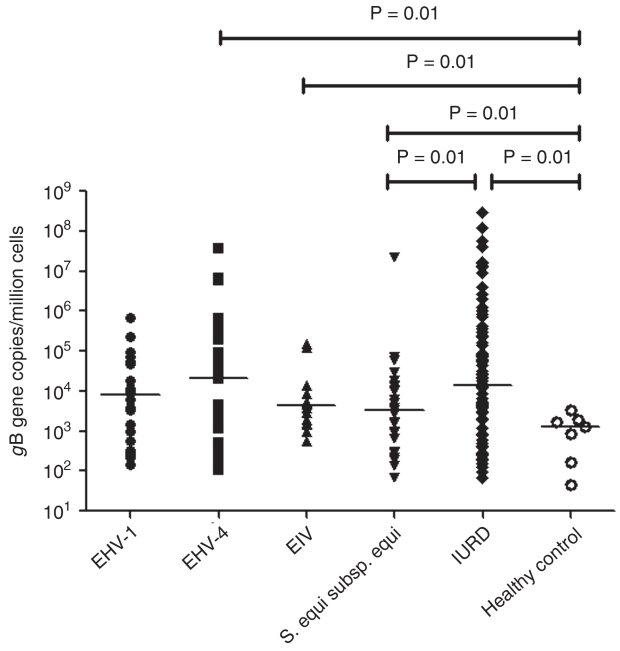

FIG 1: Quantitative results of equine herpesvirus-2 (EHV-2) detection in nasal secretions of naturally infected horses with EHV-1, EHV-4, EIV and *Streptococcus equi* subspecies *equi*, horses with signs of infectious upper respiratory tract disease, but no detectable common respiratory pathogens and healthy control horses. The results are expressed as EHV-2 *gB* gene copies per million nucleated nasal cells. Bars represent the medians for each group. Statistical significance (Wilcoxon-Mann-Whitney test) in viral loads between the different groups is represented by horizontal bars and P values

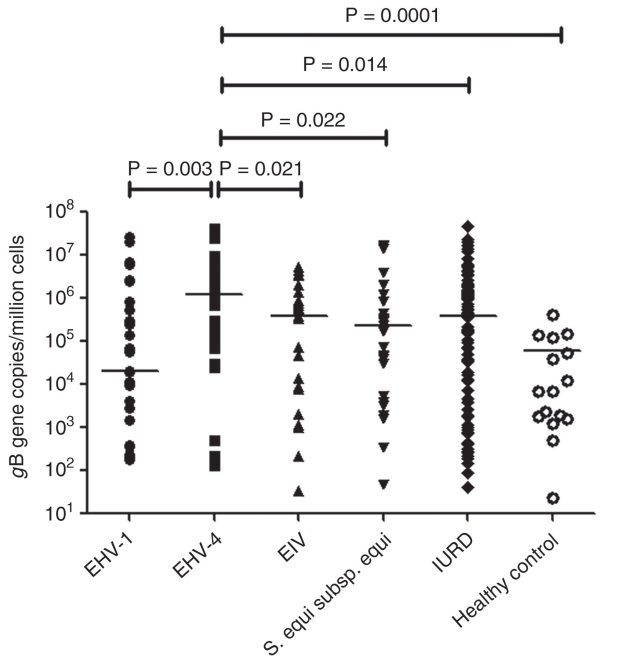

FIG 2: Quantitative results of equine herpesvirus-5 (EHV-5) detection in nasal secretions of naturally infected horses with EHV-1, EHV-4, EIV and *Streptococcus equi* subspecies *equi*, horses with signs of infectious upper respiratory tract disease but no detectable common respiratory pathogens and healthy control horses. The results are expressed as EHV-5 gB gene copies per million nucleated nasal cells. Bars represent the medians for each group. Statistical significance (Wilcoxon-Mann-Whitney test) in viral load between the different groups is represented by horizontal bars and P values

and to determine their possible association with respiratory diseases. However, this task is hampered by the fact that the pathogenicity of lesser characterised respiratory viruses is debated given that they can be recovered from both clinically affected and healthy animals (Bell and others 2006a, b; Quinlivan and others 2010).

EHV-2/-5, EAdV1, ERAV and ERBV have all been linked to acute upper respiratory disease outbreaks with fever and nasal discharge. Several studies have shown via culture, PCR or serology that these viruses are present either alone or in various combinations with common infectious respiratory pathogens, and generally affect young performance horses (Powell and others 1978, Carman and others 1997, Dynon and others 2007, Diaz-Mendez and others 2010).

Results of our study showed that lesser characterised respiratory viruses are detected at variable frequencies from the nasal secretions of horses with IURD, and to a lesser frequency in healthy control horses.

EHV-2 and EHV-5 were the most frequently detected lesser characterised respiratory viruses in the present study. Age-susceptibility to the equine gamma herpesviruses is the likely reason for the higher detection rate determined in the EHV-4 group, since this group had the lowest median age compared with the other groups. A similar hypothesis may also account for the statistical differences in EHV-5 viral loads observed between the EHV-4 group horses and the other group horses. The role of EHV-2 and EHV-5 in the nasal secretions of horses with IURD and healthy horses is still unclear. EHV-2 and EHV-5 may subvert mucosal immunity in the upper respiratory tract of horses, and therefore, increase their susceptibility to other respiratory viruses. Alternatively, infections with common respiratory pathogens may have reactivated latent EHV-2 and EHV-5 infections. This may be supported by the fact that EHV-2 and EHV-5 were less frequently detected from the nasal secretions of healthy control horses. Recent research has linked the pathogenic potential of EHV-2 and probably EHV-5 to a modulation of the host immune response (Fortier and others 2010).

EAdV1 infections have mainly been associated with respiratory disease in immunodeficient and immunocompetent foals. Further, EAdV1, similar to other adenoviruses, is likely to establish persistent infection with periods of recrudescence occurring during stressful

events (Bell and others 2006b). The number of EAdV1 PCR-positive study horses was small, and detection was only reported in horses older than one year of age. The detection of EAdV1 in these horses likely represents an incidental finding with an unlikely clinical impact.

Equine rhinitis viruses are generally considered to cause mild to severe respiratory diseases. In a previous study in Ontario using culture and serology, ERBV was found the most common cause of respiratory disease in study horses (Carman and others 1997). Of interest was the lack of detectable ERAV virus among the present study horses, while ERBV was detected in 9/336 (2.7 per cent) study horses. The difference in detection rates between studies is likely a reflection of different horse populations, knowing that exposure to equine rhinitis viruses occurs more commonly in young horses during the period of training and racing (Black and others 2007). Unfortunately, the use of the present study horses was not recorded on the submission forms. ERBV has been detected via PCR and/or culture in 1.2–30 per cent of nasal secretions collected from horses with IURD (Dynon and others 2007, Diaz-Mendez and others 2010, Quinlivan and others 2010). By comparison with these studies, the authors documented ERBV in the nasal secretions of 4/172 (2.3 per cent) IURD group horses. Concurrent infection has been previously reported between ERBV and EHV-4 and EHV-5 (Dynon and others 2007) and ERBV and EIV (Quinlivan and others 2010). To the author's knowledge, the present study is the first to report on the concurrent infection of ERBV with EHV-2 and ERBV with *S equi* subspecies *equi*.

In conclusion, the result of this study showed that EHV-2 and EHV-5 were commonly detected in respiratory secretions of horses with confirmed causes of IURD and horses with signs of IURD but no detected common respiratory pathogens. In view of the study results, it still needs to be determined if the detection of EHV-2 and/or EHV-5 in horses with IURD relates to a primary pathogenic effect, reactivation of latent stage or to modulation of the host immune response predisposing horses to coinfections. The detection of EAdV1 mainly in adult horses represents a likely incidental finding with no direct clinical impact. Although less frequently detected in the study population, ERBV appears to be an important respiratory virus associated with IURD alone, or combined with other more common respiratory pathogens.

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Notes