



# Workshop Report

## Report of the Second Havemeyer EHV-1 Workshop, Steamboat Springs, Colorado, USA, September 2008

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### Summary

**This report summarises the findings of the Second Havemeyer EHV-1 Workshop, which was held in Steamboat Springs, Colorado, USA in September 2008. A total of 38 delegates, consisting of veterinary clinicians and scientists from academia and industry participated in a series of sessions that focused on equine herpesvirus myeloencephalopathy (EHM). Each session consisted of a review, followed by short presentations on current research topics. The sessions included EHM epidemiology, *in vivo* and *in vitro* models for studying EHM, EHV-1 virulence determinants, real-time PCR diagnostics, antiviral medications and new vaccination technologies. The report summarises the key advances identified during and since the meeting. Citations are restricted to selected reviews and papers published since the workshop.**

### Introduction

Equine herpesvirus-1 (EHV-1; species *Equid herpesvirus-1*) remains a threat to equids worldwide. The virus is responsible for several clinical syndromes involving foals and mature individuals. The pathogen can cause respiratory, ocular and neurological (equine herpesvirus myeloencephalopathy; EHM) disease, and abortion in pregnant mares after the fifth month of gestation (Allen *et al.* 2004). It has long been agreed that control of these clinical syndromes requires a reduction in the magnitude and duration of the nasopharyngeal shedding of infectious virus and the cell-associated viraemia and consequent reduction in endothelial cell infection at sites of secondary viral replication. In response to these disease threats, the control of infectious disease outbreaks in the UK and USA is guided by the UK's Horserace Betting Levy Board's Code of Practice for Equine Infectious Diseases (<http://www.hblb.org.uk>, Anon 2006, 2010) and the American College of Veterinary Internal Medicine's Consensus Statement on EHV-1 (Lunn *et al.* 2009). Among these control measures, vaccination is used commonly against EHV-1. The immune response stimulated by inactivated EHV-1 within these products modulates the severity of respiratory disease with some success. Widespread vaccination

also appears to have been associated with a decline in the number of outbreaks involving multiple abortions (abortion storms), but vaccination fails to prevent abortion in individual mares. In contrast, no vaccine is licensed currently to prevent EHM, largely due to the unavailability of a suitable animal model that consistently reproduces the desired clinical disease.

Equine herpesvirus myeloencephalopathy has been described for many years, but recently there has been an apparent increase in the number of reported outbreaks in the USA involving EHM; from one in 2001 to 11 in 2006 (APHIS report; Table 1). The best known of these was a disastrous EHM outbreak in Ohio in 2003, affecting over 100 horses (Henninger *et al.* 2007). This has spurred efforts to develop an equine model of EHM to further our understanding of pathogenesis and enable vaccines to be screened for their efficacy against this disease. A key publication in recent years (Nugent *et al.* 2006) compared the DNA sequence of EHV-1 strains isolated from horses showing either neurological or non-neurological disease. This demonstrated an association between neurological disease and the presence of the nucleotide guanine (G<sub>2254</sub>) in open reading frame (ORF) 30 which translated to aspartic acid (D) at position 752 (D<sub>752</sub>) in the DNA polymerase protein. In contrast, virus isolated from horses, that did not show neurological disease possessed the nucleotide adenine (A<sub>2254</sub>) and coded for asparagine (N<sub>752</sub>). This finding has since been confirmed in North American isolates where the odds of developing EHM were much greater if the D<sub>752</sub> biovariant was isolated (Perkins *et al.* 2009). However, the 'non-neurological' virus biovariant (ORF30 A<sub>2254</sub>/N<sub>752</sub>) has been isolated from a substantial number of horses with neurological disease, suggesting that additional factors contribute to the onset of EHM. The greater pathogenicity of the D<sub>752</sub> biovariant has been elegantly demonstrated (van de Walle *et al.* 2009a). In response to the apparently increased frequency of outbreaks, EHM formed a focal point of the current meeting. As shown in Table 2, this symposium assembled an international group of EHV-1 experts from an array of disciplines to present and discuss new data on EHM in Steamboat Springs, Colorado, USA in September 2008. A notable absentee from the delegate list was Professor George P. Allen who died earlier in the year: the symposium was dedicated to his memory.

**TABLE 1: Useful sources of information relating to EHV-1 clinical disease and its management, with particular focus on EHM**

Link	Description
<a href="http://www.aphis.usda.gov/vs/nahss/equine/ehv/">http://www.aphis.usda.gov/vs/nahss/equine/ehv/</a> <a href="http://www.aphis.usda.gov/publications/animal_health/index_ah_e.shtml">http://www.aphis.usda.gov/publications/animal_health/index_ah_e.shtml</a>	USDA APHIS site on EHV-1 with several useful additional links Equine herpesvirus myeloencephalopathy: a potentially emerging disease
<a href="http://www.aphis.usda.gov/vs/nahss/equine/ehv/equine_herpesvirus_nahms_2008report.pdf">http://www.aphis.usda.gov/vs/nahss/equine/ehv/equine_herpesvirus_nahms_2008report.pdf</a>	USDA APHIS Equine Herpesvirus Myeloencephalopathy: Mitigation Experiences, Lessons Learned and Future Needs. Traub-Dargatz, J. 2009
<a href="http://www.aphis.usda.gov/vs/nahss/equine/ehv/equine_herpesvirus_brochure_2009.pdf">http://www.aphis.usda.gov/vs/nahss/equine/ehv/equine_herpesvirus_brochure_2009.pdf</a>	A guide to understanding the neurologic form of EHV infection. Also lists other useful resources. 2008.
<a href="http://www.ca.uky.edu/gluck/BiblioEHV1.asp">http://www.ca.uky.edu/gluck/BiblioEHV1.asp</a>	Gluck Equine Research Center's Bibliography of publications on EHV-1
<a href="http://www.aaep.org/vaccination_guidelines.htm">http://www.aaep.org/vaccination_guidelines.htm</a> <a href="http://www.aaep.org">http://www.aaep.org</a>	AAEP vaccination guide AAEP Practitioners' Guidelines on Infectious Disease Outbreak Control 2006 (restricted to members).
<a href="http://acvim.org">http://acvim.org</a>	American College of Veterinary Internal Medicine Consensus Statement on EHV-1
<a href="http://www.hblb.org.uk">http://www.hblb.org.uk</a> <a href="http://www.umass.edu/vetimm/equine/index.html">http://www.umass.edu/vetimm/equine/index.html</a> <a href="http://www.immunologicaltoolbox.com/">http://www.immunologicaltoolbox.com/</a>	Horserace Betting Levy Board's Codes of Practice U.S. Veterinary Immune Reagent Network Equine species site BBSRC Immunological Toolbox site

**TABLE 2: List of participants in the Havemeyer EHV-1 Workshop, arranged alphabetically**

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### EHM: the problem and the knowledge gap

The clinical signs of EHV-1 infection in horses are well described, and include biphasic fever and mild respiratory disease. However, clinical signs preceding the development of EHM in horses may include fever, hindlimb and/or scrotal oedema. Thereafter, onset of neurological disease is rapid and horses can present with ataxia, hindlimb paresis, tail hypotonia, dysuria, coprostitis, loss of perineal sensation and abortion in pregnant mares. Normally, only

a small number of horses within a group are affected. Euthanasia is necessary in approximately 30% of cases. For further information, readers are referred to reviews on this subject (Allen *et al.* 2004; Kydd and Smith 2006; Lunn *et al.* 2009; Nugent and Paillot 2009; Pusterla *et al.* 2009a). Table 1 lists useful websites that describe EHV-1 disease, information about EHM, and contemporary control strategies. Our ability to control EHV-1 is impaired by knowledge gaps in a series of critical research areas, each of which was addressed in this meeting: EHM epidemiology, *in vivo* and *in vitro* models for studying EHM, EHV-1 virulence determinants, real-time PCR diagnostics, antiviral medications and new vaccination technologies. This report summarises the key advances identified during and since the meeting. Citations are restricted to papers published since the workshop and the reviews cited above.

### Summary of key advances

1. A critical review identified the risk factors associated with an increased chance of developing EHM as: age, fever, limb oedema, stabling, season and geography.
2. The rapid onset of EHM, usually during the second week after infection, is consistent with the infection of endothelial cells and vascular compromise of the central nervous system (CNS).
3. Novel quantitative diagnostic tests have been developed to detect viral load and genotype *premortem*, aiding in more rapid diagnosis, and with the potential to predict disease outcome better (Pusterla *et al.* 2009b,c; Nemoto *et al.* 2010).
4. The frequency and epidemiology of the ORF30 D<sub>752</sub> and N<sub>752</sub> biovariants within the global equine population is complex, and requires further investigation (Allen *et al.* 2008; Perkins *et al.* 2009; Vissani *et al.* 2009; Smith *et al.* 2010).
5. The functions of the immediate early and DNA polymerase gene products and the mechanisms of viral attachment to and entry into host cells via integrins are a critical and evolving area of study (van de Walle *et al.* 2008; Breitenbach *et al.* 2009; Hasebe *et al.* 2009; Frampton *et al.* 2010).
6. An equine model of EHM using aged horses has been developed. This will aid in elucidating the pathogenesis of EHM and screening antiviral interventions (Allen 2008).
7. Animals infected with the ORF30 D<sub>752</sub> (neurological association) biovariant exhibit a greater magnitude and duration

of cell-associated viraemia and nasopharyngeal virus shedding compared with horses infected with the ORF30 N<sub>752</sub> (non-neurological association) form (van de Walle *et al.* 2009a).

8. Equine cellular and cytokine immune responses associated with protective immunity to EHV-1 are increasingly well characterised. Through the USDA-funded Veterinary Immunological Reagents Network and the BBSRC-funded Immunological Toolbox initiatives, the list of available reagents is constantly improving and includes multiplex cytokine assays and monoclonal antibodies (see Table 1) (Wagner and Freer 2009).
9. Novel experimental vaccines that limit EHV-1 cell-associated viraemia are emerging, which may now be assessed for their ability to control disease (Tsujimura *et al.* 2009; Soboll *et al.* 2010).
10. EHV-1 employs numerous diverse immune evasion strategies. Identification of the responsible genes will enhance the design of novel vaccines (van de Walle *et al.* 2009b).
11. The mechanisms and efficacy of antiviral therapies and RNA interference are under investigation, but further work is required before their clinical value can be known (Maxwell *et al.* 2008; Fulton *et al.* 2009; Garre *et al.* 2009a,b; Brosnahan *et al.* 2010).
12. The critical targets for vaccination are: 1) the prevention of infection by stimulating antibody responses in the mucosal and systemic compartments to neutralise free infectious virus; and 2) cell-mediated immunity to lyse virus infected cells, and particularly to control the cell-associated viraemia that precipitates infection of endothelial cells in the capillaries supplying the pregnant uterus and spinal cord.

### Future directions and conclusions

The field of EHV-1 research has drawn increased attention from researchers in the past decade, resulting in an accelerating pace of discovery. One of the most important goals remains the characterisation of a reliable and reproducible *in vivo* equine model of EHM in order to understand disease pathogenesis better and determine the value of novel vaccines and therapeutics. There have been considerable advances in virological assays for diagnosis and determination of pathogenicity, but assays of equine EHV-1-specific immunity continue to need better development. Additional challenging research topics include the molecular interactions of EHV-1 variants and host cells, specifically the endothelium and lymphocytes. Finally the need for an *in vitro* equine model capable of distinguishing between the neurological and non-neurological biovariants remains a priority, as this will enable experiments involving horses to be reduced and refined.

Since the previous Havemeyer EHV-1 workshop in 2004 and even since the second workshop in late 2008, a substantial volume of new information has become available about the virology, pathogenesis and epidemiology of EHV-1 disease, and prophylactic measures. Continued international collaboration within the EHV-1 community offers the best chance for progress in the fight against EHM and other manifestations of EHV-1 infection.

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## BEVA ONE-DAY WINTER CLINICAL WORKSHOP

### Dermatology

Wednesday 15th December 2010 • Marriott Hotel, Leicester

Kindly Sponsored by: Janssen Animal Health

Following the success of the dermatology sessions at BEVA Congress 2009, we are finishing the 2010 Clinical Workshop series with a one day workshop on dermatology. We think dermatology is perfectly suited to the workshop format and the expert panel will use images and supporting clinical pathology to work through a series of challenging dermatology cases.

The Dermatology Clinical Workshop is a full day of case-based interactive discussion led by our panel of experts using electronic voting to facilitate delegate interaction. The four sessions will provide a unique opportunity for delegates to ask questions, contribute to discussion and see how the experts work through problems. To ensure delegates get real value from the day, the cases that will be discussed will be available on the BEVA website in advance of the workshop to enable you to review the case material. Once registered, you will be able to submit your own questions, images and cases to the panel (either in advance or on

the day) so that we can tailor the workshop to your own areas of interest and also help you by having the panel discuss your cases. The final session of the day will incorporate clinical material and questions submitted by you, the delegates, to ensure the workshop covers material relevant to you. If you are interested in dermatology, or want to brush up on your dermatology skills, we are certain you will not find a better opportunity anywhere in Europe in 2010.

#### Chair:

Professor Josh Slater BVM&S PhD DECEIM MRCVS Hertfordshire

#### Speakers:

Mr Rob Pilsworth MA VetMB BSc CertVR MRCVS Suffolk  
Mr Andy Durham BSc BVSc Cert EP DEIM Dip ECEIM MRCVS Hampshire  
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