clinical importance of the gammaherpesviruses in horses remains undetermined. This study aimed to determine the prevalence, and any association between equine respiratory herpesviruses EHV1, -2, -4 and -5 infection in horses with and without clinical signs of respiratory disease. Cases were those horses that were presented for exclusion of equine influenza virus (EV) that exhibited signs of respiratory disease including pyrexia, nasal discharge, and/or coughing during the 2007 Australian EV outbreak. Nasal swabs were taken from 407 horses in Victoria, which remained free of EV and included clinically normal horses that had been screened for regulatory purposes. Samples were placed in viral transport media and subsequently stored at -80°C. Quantitative PCR was performed using primers specific for EHV1, -4, -2 and -5. A multiplex Taqman assay was performed with labelled probes for the alphaherpesviruses EHV-1 and -4. Separate individual assays for the gammaherpesviruses EHV-2 and -5 were also performed. Of the three horses detected shedding EHV-1, and the five shedding EHV-4 only one was noted to have clinical signs referable to respiratory disease. The proportion of EHV5 infected horses in the diseased group (85/120, 70.8%) was significantly greater than those not showing signs of disease (137/249, 55%). The odds of EHV-5 positive horses demonstrating clinical signs of respiratory disease were twice that of EHV-5 negative horses (OR 1.99, 95% CI 1.25 to 3.16). Horses infected with EHV-2 made up a smaller proportion in the diseased group (18/120, 15.0%) compared to those without disease (61/249, 24.5%; P=0.042). The odds of disease in EHV-2 positive horses were approximately half that in EHV-2 negative horses (OR 0.54, 95%CI 0.31 to 0.97) Fifty of the 83 horses (60.2%) shedding EHV-2 were also shedding EHV5, however there was no greater likelihood of EHV5 detection in these horses compared to those without detectable EHV-2 (199/324, 61.4%; P=0.90). The dual infected horses were no more likely to exhibit signs of disease (14/46, 30.4%) than those shedding EHV-2 alone (4/33, 12.1%; P=0.063) or EHV-5 alone (71/176, 40.3%; P=0.24). Logistic regression showed no interaction between EHV-2 and -5 shedding on clinical signs of respiratory disease (P=0.41). No quantitative difference between mean loads of EHV shedding between diseased and non-diseased horses was able to be detected. The clinical significance of respiratory gammaherpesvirus infections in horses remains to be determined, however this survey adds to the mounting body of evidence associating EHV5 with equine respiratory disease.

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090 Equine PBMC cytokines profile and efficacy of a Parapoxvirus ovis based-immunomodulator after in vitro α- and γ-EHV infection


1 LABÉO Frank Duncombe, 14053 Caen, France; 2 Normandie Université, 14000 Caen, France; 3 Unité Recherche Risques Microbiens (U2RM), EA 4655, 14032 Caen, France; 4 Hippolia Foundation, 14000 Caen, France; 5 Centre d’Etudes et de Recherche sur le Médicament de Normandie, Université de Caen Normandie, 14000 Caen, France; 6 Anses, Université Paris-Est, Laboratoire de Santé Animale, UMR 1161 Virologie, Maisons-Alfort, 94700 France; 7 Anses, Laboratoire de pathologie Equine, Unité Virologie, 14430 Goussainville, France; 8 Institut Pasteur, Unité de Chimie et Biocatalyse, 75015 Paris France; 9 CNRS UMR 8601, Université Paris Descartes, 75006 Paris, France

In virology, different conventional methods are commonly used for virus titration and infectivity studies. Most of them are based on direct examination of viral cytopathic effect, which is time consuming, tedious and requires an endpoint reading. The RTCA xCelligence system (ACEA Biosciences) has been developed to follow cellular events and their dynamics in real time using a micro-electrical biosensor technology. The aim of this study was to evaluate the feasibility of using this biosensor technology to measure the effect of different equid herpesviruses on equine dermal cell cultures, with or without antiviral acyclovir treatment. Cells were infected with EHV-1 (VR700), EHV-4 (VR2230), EHV-2 (VR701) or EHV-3 (VR352). The viral effects were monitored by 3 different methods: impedance measurements (RTCA), cytopathic effects recording (microscopy) and viral load quantification (qPCR). The RTCA technology showed a dose dependent drop of the cellular index induced by specific impedance variations measured during growth of each virus tested. These results correlated with the cytopathic effect induced by the different herpesvirus species, as well as viral loads measured by qPCR. For example, after 3 days of incubation with EHV-1, RTCA results showed an increase of the cellular index from 0% (infection
without antiviral) to 45% and 100% in presence of Acyclovir (10µl/mL and 100 µg/mL, respectively). These results are correlated with the decreased of viral load measured in the presence of Acyclovir: 10¹⁰ genome copies/mL with EHV-1 only, 10⁷ copies/mL with EHV-1 + Acyclovir 10 µg/mL and 10³ copies/mL with EHV-1 + Acyclovir 100 µg/mL. In conclusion, the cellular impedance monitoring system is a new real-time method to monitor cell growth and viral proliferation in cell cultures. This technology is well adapted for high-throughput screening of antiviral molecules. In fact, the xCELLigence system enables continuous quantification of cell adhesion, proliferation, cell death and detachment during viral infection.

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057 Genetic variation and dynamics of infections of equine herpesvirus type 5 (EHV-5) in individual horses

Helena Back², Karin Ullman¹, Mikael Leijon¹, Robert Söderlund², Johanna Penell³, Karl Ståhl³, John Pringle⁵, Jean-François Valarcher¹, François Valarcher¹

¹Department of Virology, Immunobiology and Parasitology, National Veterinary Institute, Uppsala, Sweden; ²Department of Bacteriology, National Veterinary Institute, Uppsala, Sweden; ³Department of Veterinary Epidemiology and Public Health, University of Surrey, Guildford, UK; ⁴Department of Disease Control and Epidemiology, National Veterinary Institute, Uppsala, Sweden; ⁵Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

*shared first authorship

Equine gamma herpesvirus type 5 (EHV-5) is related to the human Epstein–Barr virus (EBV) and has frequently been observed in equine populations worldwide. EHV-5 was previously assumed to be low to nonpathogenic, however, studies have also related the virus to the severe lung disease equine multinodular pulmonary fibrosis (EMPF). Genetic information of EHV-5 is scanty, where the whole genome was recently described and only limited nucleotide sequences are available. In this study, partial of the EHV-5 glycoprotein B (gB) gene were analyzed in nine horses by using next generation sequencing (NGS). The included samples were from eight healthy Standardbred trotters at the same professional training yard that were sampled twice with one year apart, and from one horse diagnosed with EMFP from which samples were taken pre and post mortem. The analysis resulted in obtaining of 27 partial gB gene sequences, 11 unique sequence types and 5 amino acid sequences. These sequences could be classified within four genotypes (I-IV) of EHV-5 gB gene based on the degree of similarity of the nucleotide and amino acid sequences, and in this work horses were shown to be identified with up to three different genotypes at the same time. The observations showed a range of interactions between EHV-5 and the host over time, where the same virus persists in some horses whereas others have a more dynamic infection pattern including strains from different genotypes. This study provides insights into genetic variation and dynamic of EHV-5 and highlights that further work is needed to understand the EHV-5 interaction with its host.

Poster

154 Detection of equine herpesvirus-2 and -5 infection in asymptomatic horses from Brazil

AM Dall Agnol¹, E.A. Beuttemmüller¹, D. Pilz¹, M.V. Oliveira¹, S.A. Headley², L.E.S. Ferraz³, A.F. Alfieri¹, A.A. Alfieri¹

¹Laboratory of Animal Virology and Multi-User Animal Health Laboratory, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Londrina, Paraná, Brazil; ²Laboratory of Animal Pathology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Londrina, Paraná, Brazil; ³Laboratórios Vencofar - do Brasil, Londrina, Paraná, Brazil

Infections caused by equine gammaherpesvirus 2 and 5 usually occur during the early stages of the life of horses, followed by periodic reactivation of a latent infection. The occurrence of equine herpesvirus 2 (EHV-2) is related with immunosuppression and has been associated with outbreaks of respiratory disease. Alternatively, equine herpesvirus 5 (EHV-5) has tropism for the respiratory tract and has been related to equine multinodular pulmonary fibrosis. Since the presence of these viruses was never reported in Brazil, the aim of this study was to detect EHV-2 and -5 in Brazilian horses. Twenty-six nasal swabs were collected from horses without signs of respiratory distress from two distinct pure-breed farms (A, n=18; B, n=8). Nucleic acid purification was performed by using a combination of the phenol/chloroform/isoamyl alcohol and silica/guanidium isothiocyanate methods. The identification of EHV-2 and -5 was done by using a nested PCR assay that targeted the gB gene. The PCR results were confirmed by sequencing using ABI 3500 Genetic Analyzer. From Farm A, 5 nasal swabs were positive for EHV-2, 10 contained EHV-5 DNA, and one sample had the DNA of both viruses. From the second farm, 7 samples were positive for EHV-2, 6 for EHV-5, and 6 contained the DNA of both viruses. In summary, from a total of 26 nasal swabs, 46.1% (12) were contained EHV-2 DNA, 61.5% (16) were positive for EHV-5, and 26.9% (7) had the DNA of both viruses. This was the first detection of equine gammaherpesviruses 2 and 5 in horses from Brazil. These results obtained from asymptomatic horses suggest that these viruses are probably endemic in Brazil, similarly as previously reported in Europe, USA and Australia.

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219 Sequence and recombination analyses of archived field isolates of equine herpesviruses 1 and 4

P.K. Vaz¹, J. Horsington¹,², C.A. Hartley¹, G.F. Browning¹, N.P. Ficorilli¹, M.J. Studdert¹, J.R. Gilkerson², J.M. Devlin¹

¹Asia-Pacific Centre for Animal Health, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia; ²Present address: Australian Animal Health Laboratory, CSIRO, 5 Portarlington Rd, East Geelong, Victoria, 3220, Australia; ³Centre for Equine Infectious Diseases, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia

Recombination allows evolution to occur in viruses that have an otherwise stable DNA genome with a low rate of nucleotide substitution. High-throughput sequencing of complete herpesviral genomes has recently allowed field recombination to