MEGA6 software. The molecular modeling was performance in I-Tasser online server and, the structure were analyzed with UCSF Chimera package. The phylogenetic analyses showed that the Asian EIV population has a geographic division. All Japanese strain grouped in Florida Clade 1 next to USA strains. The EIV population from mainland (China, Mongolia, Kazakhstan and India) belong to Florida Clade 2. Furthermore, a geographic association is observed. Two EIV population can be shown Indian strains corresponding to 2008 and 2011 EI outbreak. In China, the 2007-2008 strains from a monophyletic cluster. Interesting, the strain Mongolia/56/2011 evolve to the Mongolia 2011 outbreak and, similar strain were detected in 2012 in Kazakhstan (South Kazakhstan/236/2012). The strains detected in China in 2013 (Heinlongjian/SS1/2013 and Xuzhou/01/2013) were linked with the strains detected in Mongolia in 2012. Mapping the amino acid (aa) substitution, four differences (V78A, S159N, S162P and K189Q) are found in Huabei/1/2007 respect to the vaccine strain Ibaraki/1/2007, three in the antigenic site B (top of the protein) and one in E. Comparing the vaccine strain against Heinlongjian/SS1/2013, two extra aa substitution can be seen (A144T and E198G). Our study support the understanding of the molecular evolution of H3N8 EIV in Asia and will facilitate future investigations of the epidemiology of these viruses because, the EIV strains still evolving in the population. Furthermore, contributes to the monitoring of vaccines the currently available.

Posters

042

Optimisation of equine influenza pseudotyped virus production

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Pseudotyped viruses (PVs) provide a flexible, safe platform for fundamental virological studies and antibody/antiviral screening assays. Generation of influenza PVs involves co-transfection of producer cells with plasmids encoding the necessary viral components. The pseudotype virus neutralization assay (PVNA) is a sensitive technique to measure protective antibody responses. Furthermore, many traditional methodologies (e.g. HI, SRH) detect only surface glycoprotein binding antibodies whereas the PVNA quantifies infectivity-neutralizing responses. Two enzymes crucial for the infectivity of influenza viruses are; HA-cleaving cellular proteases and neuraminidase (NA). Transmembrane protease serine 2 (TMPRSS2) has been shown to cleave group 2 influenza HAs in both a native and PV context, including an equine H3 PV. However the efficacy of alternatives, such as TMPRSS4 and human airway trypsin-like protease (HAT), has not been tested. In nature, influenza NA cleaves sialic acid permitting viral egress. When generating PVs, an exogenous source of NA (often from *Clostridium perfringens*) is typically added for cleavage. However, an endogenous source of NA (i.e. NA encoding plasmid) has rarely been used in the transfection mix to produce influenza PVs and not yet trialled for H3. Producing high titre PVs is important as this permits minimal quantities to be used in PVNAs, and repeat experiments can be carried out using the same batch of virus, minimising intra-study variability. Here we investigated the role of different proteases and NAs to optimise the generation of PVs. Initially, influenza PVs were generated via co-transfection of HEK293T/17 cells with plasmids expressing the A/equine/Richmond/1/2007 influenza H3 HA, HIV gag-pol, firefly luciferase reporter and test endoprotease (with the standard addition of exogenous NA). The nature and quantity of protease plasmid affected PV titre and so the optimum was subsequently used in a five plasmid co-transfection incorporating a different NA plasmid representative of N1-4, 8 and 9 subtypes. Some, but not all, NA subtype plasmids enabled generation of PVs with H3 HA and there were notable differences in production titre. PVs were then taken forward into PVNAs, to determine whether the presence of NA in the PV envelope influences antibody neutralization. Results showed that neutralization was not impeded by the presence of NA.

070

Production and establishment of a new candidate horse antiserum (Common OIE International Standard/ European Pharmacopoieia Biological Reference Preparation) to the Florida Clade 2 equine influenza virus A/eq/Richmond/1/07

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Equine influenza (EI) is a major respiratory disease of the horse caused by the highly infectious equine influenza virus (EIV), outbreaks of which induce significant welfare and economic consequences. EI vaccination is one of the most effective methods of prevention, with diverse EI vaccines commercially available worldwide. As for all influenza viruses, EIV constantly evolves through antigenic drift in order to evade natural and vaccine immunity. Genetic and antigenic variations are monitored by the OIE (World Organisation for Animal Health) Expert Surveillance Panel for EI vaccine composition (OIE ESP) in order to maintain optimal EI vaccine protection. In 2010, the OIE ESP recommended that EI vaccines should contain EIV strains from both Florida Clade 1 and Clade 2 sublineages (FC1 and FC2, respectively). In this context, up-to date reference antiserum standards are essential for normalisation of immunogenicity assays, such as the single radial haemolysis assay (SRH) and the haemagglutination inhibition (HI) test, for EI vaccines batch potency assay and efficacy evaluation in clinical trials, and for diagnostic tests. A biological reference preparation (BRP) equine antiserum to the FC1 EIV strain A/eq/South Africa/4/03 is currently available, but none exist for FC2 EIV strains. This FC2 BRP is urgently required. Study aims: to produce and establish a new candidate equine antiserum specific to the EIV strain A/eq/Richmond/1/07 (FC2 representative strain) asan European Pharmacopoeia (*Ph. Eur.*) BRP and as an OIE-approved International Standard. Objectives & Results: 1) BRP production: two Welsh mountain ponies, seronegative for EIV, were successfully immunised against the EIV strain A/eq/Richmond/1/07 by experimental infection. A total of 1.230 litre of serum was collected from day 14 to day 23 post infection and titrated by SRH using the EIV strain A/eq/Richmond/1/07 as antigen. SRH antibody titres ranged from 144mm^2 to 217mm^2. 2) BRP titration and validation: after pooling and inactivation with Beta-propiolactone, a batch of BRP and SRH/HI antigens will be provided to up to 10 independent collaborative laboratories for SRH and HI titration. Individual titrations will be analysed by the EDQM statistics department in order to define the new FC2 BRP potency titre. Conclusion: the availability of up-to date reference standards is essential to develop, evaluate and standardise the different methods of prevention against EI (e.g. EI vaccines and serological diagnostic tools). This presentation aims to review the multi-stage process of production and establishment of a new horse antiserum BRP specific to the FC2 representative EIV strain, with results currently obtained highlighted and pivotal stages presented and discussed.