View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector



broader immune response. This work describes the generation of two immunogens based on the stem region of HA from the A/Equine/Argentina/93(H3N8) influenza strain: a recombinant protein expressed in prokaryotic cells (ΔHAp), and a DNA vaccine (ΔHAe). A DNA fragment encoding ΔHAp was cloned into the pET22b vector, and the recombinant protein was purified from inclusion bodies. The ΔHAe coding sequence was cloned into eukaryotic vectors that were transfected in HEK 293T cells. The expression of proteins was determined by Western blot assay. The immunogenicity of both vaccine candidates was studied using different combination of antigens in mice. Immunizations were done two times with a three-week interval. The DNA vaccine was given intramuscularly and the recombinant protein was given intraperitoneally in incomplete Freund's adjuvant (Sigma). A group of five mice received two immunizations with 50 ug of DNA vaccine; a second group received two immunizations with 10 ug of recombinant protein; a third group was primed with 50 μg of DNA vaccine and boosted with 10 ug of recombinant protein; a fourth group was primed with 10 ug of protein and boosted with 50 μg of DNA vaccine; and a fifth group received only PBS. We found that immunization with two doses of recombinant protein was more immunogenic that vaccination with DNA only or combination of DNA and protein. In order to analyze cross-reactivity of the immune sera, ELISA plates were coated with 4 HA units of influenza strains belonging to groups 1 (H1N1) and 2 (H3N2). Plates coated with recombinant protein were used as a positive control. Higher antibody titers against viruses belonging to the same phylogenetic group were observed when mice were immunized with recombinant protein. However antibody titers were broader and reacted against viruses belonging to different phylogenetic groups when mice were immunized with DNA. Conclusions: Recombinant proteins were expressed correctly in prokaryotic and eukaryotic systems. Homologous antibody titers were higher in animals immunized with recombinant proteins, while DNA immunization induced a heterotypic immune response.

## 116
## Protective efficacy of inactivated EIV vaccine (H3N8) in BALB/c mice model

Selvaraj Pavulraj [1], Nitin Virmani*[1], Bidhan C. Bera [1], Alok Joshi [2], Taruna Anand [1], Meenakshi Virmani [3], Rajesh Kumar Vaid [1], Raj K. Singh [4], B.N. Tripathi [1]
[1] *ICAR- National Research Centre on Equines, Hisar, Haryana, India;* [2] *Veterinary Hospital, Naini, Barakot, Uttarakhand - 263601, India;* [3] *Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana;* [4] *Indian Veterinary Research Institute, Bareilly, UP, India*

Equine influenza (EI) is highly contagious acute respiratory disease of equines caused by Influenza A virus belong to the family *Orthomyxoviridae*. Like other influenza viruses, equine influenza virus (EIV) show periodic antigenic drift that leads to vaccines strains becoming redundant. This necessitates regular harmonization of vaccines and strain substitution. The studies in large animals for potency testing is difficult and suitable small animal model is required for testing the protective efficacy of the novel or updated EI vaccine candidates prior to final testing in equines. The present investigation focuses on use of BALB/c mice for studying the protective efficacy of inactivated indigenous H3N8 vaccine subsequent to challenge with EIV. BALB/c mice immunized with inactivated EI vaccine were challenged through intranasal instillation of EIV @ $2x10^{6.24}$ $EID_{50}$ in 20ul volume. Mice were monitored for humoral immune response kinetics (HAI), clinical examination, virus isolation, qRT-PCR, histopathology and immunohistochemistry (IHC). Immunized mice showed HAI titre of 138.67±25.69 after two boosters. Clinically unvaccinated mice suffered severe respiratory disease and showed respiratory distress, forced expiration, ruffled coat, reduced activity and crouching at corners at 2-7 days post challenge (dpc) with 6.34±0.21 % weight reduction at 5 dpc, whereas vaccinated mice showed minimal signs at 2-4 dpc with 3.69±0.13% weight reduction at 2 dpc. Hematology showed mild leucopenia with lymphopenia at 7 dpc and lymphocytosis at 3 dpc in unvaccinated and vaccinated mice, respectively. Gross lesions in unvaccinated mice after challenge were congestion of nasal mucosa, grayish mucinous exudate in trachea, severe consolidation (3-4 mm × 2-3 mm) of lung parenchyma with congestion and gray discoloration at 2-5 dpc. In comparison, vaccinated mice showed only congestion of lung parenchyma without consolidation at similar intervals. Main histopathological changes *viz.* rhinitis, tracheitis, necrosis of bronchi and bronchiolar epithelium, peribronchitis and perivascular cuffing of neutrophils and lymphocytes, diffuse interstitial pneumonia were restricted to respiratory tract in unvaccinated mice at 3-5 dpc. Vaccinated mice developed mild respiratory lesions which resolved rapidly (2-3dpc). Gross and histopathological scoring revealed that vaccinated mice developed fewer lesions than unvaccinated mice upon challenge. Unvaccinated and vaccinated mice showed similar EIV antigen distributions in nasal turbinate, trachea and lung, but intensity and duration of positivity was less in vaccinated mice. Virus isolation from nasal washings and lung tissues showed less virus shedding (1.25 $log_{10}$ $EID_{50}$/ml) and early clearance (1 dpc) in vaccinated than unvaccinated mice (5.25 $log_{10}$ $EID_{50}$/ml at 1 dpc and persisted up to 5 dpc). Further, qRT-PCR showed unvaccinated mice shed significantly more virus in nasal washings and lungs up to 5 dpc as compared to vaccinated mice which shed very less virus up to 3 dpc. Thus, it can be concluded from the present investigations that BALB/c mice is a potential small animal model for testing EIV vaccine candidates prior to testing in final host. Findings of the study will be discussed in detail during the presentation.

## 123
## Molecular evolution of H3N8 EIV in China, phylogenetic and structural analyses

S. Miño [1,2], T. Qi [1], W. Guo [1], X. Wang [1]
[1] *National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, PR China;* [2] *Instituto de Virología, CICVyA, INTA Castelar, Las Cabañas y De los Reseros s/n, (1686), Hurlingham, Buenos Aires, Argentina*

Equine influenza virus (EIV) belongs to the Orthomyxoviridae family and is responsible for the severe acute respiratory disease of equine influenza (EI) in the horse population. Two genotypes of EIV have been isolated, H7N7 (considered extinct) and, H3N8 that has diverged into American and the European lineages. The American lineage further evolves into the Kentucky, South American, and Florida clade 1 and 2. Currenly, Florida Clade 1 and 2 are the most prevalent genotypes worldwide. In China, four main EI epizootic events (1970, 1989, 1994 and 2007-2008) occurred. The analyses showed that all EIV were related and clustered in a single branch belonged to Florida Clade 2. The strains present in the vaccine are SouthAfrica/1/2003 and Ibaraki/1/2007 and, although vaccination is currently implemented in China, sporadic even of EI were detected during the last years. The aim of this work is analyze the EIV detected during 2011, 2013 and 2015 in China based on phylogenetic and structural analyses of hemagglutinin protein.The Data set were constructed by BLAST analyses with Huabei/1/2007 strain to defined sequences to compared. One hundred and two HA sequences available in GenBank were used. The phylogenetic analyses was carried out in

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

R. Kinsley*[1], F. Ferrara[1], N. Temperton[1], S. Pöhlmann[2], Simon Scott[1]

[1] *Viral Pseudotype Unit, School of Pharmacy, University of Kent, Central Avenue, Chatham Maritime, ME4 4TB, UK;* [2] *German Primate Centre, Leibniz Institute for Primate Research, 37077 Göttingen, Germany*

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

R. Paillot*[1], M.R. Lopez-Alvarez[1], D. Garrett[1], I. Birand[1], M.-E. Behr-Gross[2]

[1] *Animal Health Trust, Newmarket, United Kingdom;* [2] *European Directorate for the Quality of Medicine and Healthcare (EDQM), Council of Europe, Strasbourg, France*

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.