4TH INTERNATIONAL SYMPOSIUM ON NEGLECTED INFLUENZA VIRUSES

PROGRAMME BOOK

BRIGHTON, UK
18-20 APRIL 2018

Organised by:
The International Society for
Influenza and other Respiratory Virus Diseases
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Neglected Influenza Viruses Group

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WELCOME

We are delighted to welcome you to Brighton, UK for the 4th International Symposium on Neglected Influenza Viruses.

This symposium will retain the format, and build on the success of the previous symposia held in 2010, 2013 and 2015. Together, we will explore the latest surveillance data, vaccination and control strategies, diagnostic techniques, experimental research data and epidemiological and economic impact studies relating to swine, equine, canine and other non-human/non-avian viruses in the family *Orthomyxoviridae*.

Our goal is to remove professional barriers and extend the boundaries – sharing our knowledge of these viruses across continents and disciplines. We wish to promote a trans-disciplinary, co-ordinated approach to the control of influenza, integrating veterinary, scientific and medical input to protect human and animal health.

We are extremely grateful to our generous sponsors without whose support; it would not have been possible to hold this meeting. We are thankful to those on the Scientific Committee who designed the programme, raised funds, recruited speakers and reviewed abstracts.

Thank you for joining us in Brighton. We hope that the symposium inspires you, leads to fruitful future collaborations and energises you in your pursuit of understanding these fascinating and challenging viruses.

Yours sincerely,

Dr Janet M. Daly BSc PhD SFHEA FRCPath
Associate Professor in Emergent Viruses
School of Veterinary Medicine & Science
University of Nottingham
UK

Dr Nicola S. Lewis BSc BVetMed PhD MRCVS.
Deputy Director of EU/OIE/FAO International
Reference Laboratory for Avian Influenza,
Newcastle Disease and Swine Influenza
Animal and Plant Health Agency (APHA) - Weybridge
UK

SCIENTIFIC COMMITTEE

Janet Daly, Chair	University of Nottingham UK
Nicola Lewis, Co-Chair	University of Cambridge UK
Tom Chambers	University of Kentucky USA
Ann Cullinane	Irish Equine Centre Ireland
Todd Davis	CDC Atlanta, USA
Susan Detmer	University of Saskatchewan Canada
Gregory Gray	Duke University Medical School USA
Takehiko Saito	National Institute of Animal Health, National Agriculture and Food Research Organization(NARO) Japan
Stacey Schultz-Cherry	St Jude Children's Research Hospital USA
Gaëlle Simon	Swine Virology Immunology Unit Anses France
Nitin Virmani	National Research Centre on Equines India
Christopher Hamilton-West	University of Chile, Santiago Chile

PROGRAMME

Wednesday, 18 April 2018

4:00 – 6:00 pm *Registration*

6:00 – 8:00 pm *Welcome Reception*

Thursday, 19 April 2018

8:00 – 12:00 pm *Registration*

8:30 – 8:45 am **Welcome and Opening Remarks**
Janet Daly and Gregory Gray

8:45 – 9:30 am **Keynote:** #NegFluMatters - Flu ecology is inclusive
Jonathan Runstadler**,** Tufts University, USA

9:30 – 12:00 pm **Session I: Virus Transmission and Control**
Conveners: **Tom Chambers, Janet Daly and Gaëlle Simon**

9:30 – 9:45 am Assessment of zoonotic transmission of swine influenza A viruses to naive or vaccinated ferrets
Sharon M. Brookes
Animal and Plant Health Agency-Weybridge, UK

9:45 - 10:00 am Differential immunogenicity of various heterologous prime-boost vaccine regimens using swine and human H3N2 influenza viruses in pigs
Sharon Chepkwony
Faculty of Veterinary Medicine, Ghent University, Belgium

10:00 - 10:15 am Neutralizing antibody response to booster/priming immunization with new equine influenza vaccine in Japan
Takashi Yamanaka
Equine Research Institute, Japan Racing Association, Japan

10:15- 10:45 am **AM Coffee Break**

10:45 - 11:00 am A reassortant swine influenza A virus incorporating genes from pandemic (H1N1) 2009 and swine subtype H1N2 viruses is capable of interspecies transmission in pigs and ferrets
Helen E. Everett
Animal and Plant Health Agency-Weybridge, UK

11:00 - 11:15 am Antibody landscaping in the context of vaccination against influenza A viruses in European swine
Sara Lopes
Department of Zoology, University of Cambridge, UK

11:15 - 11:30 am Novel determinants of equine influenza virus morphology
Pablo Murcia
MRC Centre for Virus Research, University of Glasgow, UK

11:30 - 11:45 am — Impact of HA stability on influenza virus replication and transmission in swine
Charles J. Russell
St. Jude Children's Research Hospital, Memphis, USA

11:45-12:00 pm — Dynamics and parameters of influenza A virus transmission in nursery pigs
Montse Torremorell
College of Veterinary Medicine, University of Minnesota, USA

12:00 - 1:30 PM BUFFET LUNCH

1:30 – 4:45 pm Session II: Surveillance and Disease Investigation
Conveners: **Gregory Gray and Nicola Lewis**

1:30 – 2:00 pm — **Invited Speaker:** Investigating the epidemiology of swine-to-human influenza A virus transmission at agricultural fairs
Andrew Bowman, Ohio State University, USA

2:00 - 2:15 pm — Antigenic evolution of global swine influenza A viruses and pandemic risk
Divya Venkatesh
University of Cambridge, UK

2:15 - 2:30 pm — Pathogenesis and transmission of influenza A viruses with dominant H1 genome constellations found in US swine herds
Carine K. Souza
National Animal Disease Center, USDA-ARS, USA

2:30 - 2:45 pm — Triple reassortant H3N2 with seasonal human H3, pandemic internal genes and N2 of swine origin circulates in Danish swine herds
Lars Erik Larsen
National Veterinary Institute, Technical University of Denmark, Denmark

2:45 - 3:00 pm — Influenza A surveillance in the pig population of Great Britain (1991-2017)
Sharon M. Brookes
Animal and Plant Health Agency-Weybridge, UK

3:00 - 3:15 pm PM Coffee Break

3:15 - 3:30 pm — Genetic and antigenic diversity among recently identified human-like A(H3N2) variant viruses detected in the USA
Joyce Jones
Centers for Disease Control and Prevention, USA

3:30 - 3:45 pm — Improved surveillance using primary respiratory epithelial cells
Stacey Schultz-Cherry
St Jude Children's Research Hospital, Memphis, USA

3:45 - 4:00 pm — Surveillance of swine influenza A, B, C and D viruses in Europe 2015-2017
Dinah Henritzi
Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Germany

4:00 - 4:15 pm — The other influenza surface glycoprotein: Probing the antigenic differences between N2 neuraminidase lineages of North American swine influenza A viruses
Bryan S. Kaplan
National Animal Disease Center, USDA-ARS, USA

4:15 - 4:45 pm **Invited Speaker:** Isolation and characterization of a novel influenza A virus from Rousettus Aegypticus bats
Ghazi Kayali, Human Link, Lebanon

4:45 - 6:30 pm Poster Session and Cocktail Reception

6:30- 8:00 pm BUFFET DINNER

Friday 20th April 2018

8:30 – 12:00 pm Registration

8:45 – 09:30 am Keynote: Mechanisms and consequences of influenza A virus host switching
Wendy Barclay, Imperial College London, UK

09:30 – 12:00 pm Session III: Clinical and Experimental Virology
Conveners: **Stacey Schulz-Cherry, Susan Detmer and Nitin Virmani**

09:30 – 10:00 am **Invited Speaker**: Development/update of equine influenza vaccines: a summary of some of the clinical studies and requirements for registration (and some unexpected events)
Romain Paillot, LABEO Frank Duncombe – UniCaen (BioTARGEN), France

10:00 – 10:15 am Development of an in vitro model to study bacterial infection secondary to influenza A virus
Janet M Daly
School of Veterinary Medicine and Science, University of Nottingham, UK

10:15 - 10:30 am Evolutionarily distinct influenza A viruses show different gene regulation profiles in equine cells
Julien Amat
MRC - University of Glasgow Centre for Virus Research, UK

10:30– 10:45 am Evaluation of equine influenza virus neutralising antibody responses induced by vaccination using a pseudotype virus based assay
Simon Scott
Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent, UK

10:45- 11:00 am AM Coffee Break

11:00 - 11:15 am The bat influenza H17N10 is neutralized by broadly-neutralizing monoclonal antibodies and its neuraminidase facilitates viral egress
Nigel Temperton
Medway School of Pharmacy, University of Kent, UK

11:15 - 11:30 am In vivo evaluation of monovalent vaccines against challenge with a contemporary alpha H1N2 influenza A virus in swine
Susan Detmer
University of Saskatchewan, Canada

11:30 - 11:45 am Novel vaccines against Swine Influenza A virus
Pauline M. van Diemen
Animal and Plant Health Agency-Weybridge, UK

11:45 - 12:00 pm Transmission of influenza A virus from nurse sows to adopted piglets during lactation
Jorge Garrido Mantilla
College of Veterinary Medicine, University of Minnesota, USA

12:00 - 1:30 PM BUFFET LUNCH

1:30 – 3:45 pm Session IV: Emerging Issues and New Developments
Conveners: **Ann Cullinane, Todd Davis and Taki Saito**

1:30 – 2:00 pm **Invited Speaker:** Surveillance and pathogenesis of the recently identified influenza D virus
Mariette Ducatez, École Nationale Vétérinaire de Toulouse, France

2:00 - 2:15 pm Attachment of IDV to the respiratory tract of cattle, small ruminants, swine and horse: a call for increased surveillance
Eva Mazzetto
Istituto Zooprofilattico Sperimentale delle Venezie, Italy

2:15 – 2:30 pm Mab-based competitive ELISA for the detection of antibodies against influenza D virus
Ana Moreno
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy

2:30 – 2:45 pm Isolation and characterization of equine-like H3 viruses from wild birds in Chile
Stacey Schultz-Cherry
St Jude Children's Research Hospital, Memphis, USA

2:45 – 3:00 pm Outbreaks of influenza of swine and human origin in mink (Neovison vison)
Charlotte Kristiane Hjulsager
National Veterinary Institute, Technical University of Denmark, Denmark

3:00 – 3:15 pm PM Coffee Break

3:15 – 3:30 pm H7N2 feline influenza virus evaluated in a poultry model
David L. Suarez
Southeast Poultry Research Laboratory, USDA, USA

3:45 – 4:00 pm The evolution and epidemiology of H3N2 canine influenza virus in the USA and Asia
Colin R. Parrish
Cornell University, USA

4:00 – 4:30 pm **Invited Speaker:** Evolution of Swine Influenza Viruses in China
Huachen Zhu**,** University of Hong Kong

4:45 – 5:00 pm Meeting Summary, Prize Presentation and Close
Janet Daly and Nicola Lewis

6:30 pm – Optional Dinner

INVITED SPEAKERS

Keynote 1: #NegFluMatters - Flu ecology is inclusive

Jonathan Runstadler, *Cummings School of Veterinary Medicine at Tufts University, USA*

At the time of the 1918 influenza pandemic 100 years ago, the ecology of influenza was likely different in significant ways than it is in most parts of the world today. However, disease ecology is a fluid environment and significant changes in both the natural and human environment are likely to continuously impact viral ecology in significant ways that depend on the geographic location as well as anthropogenic impacts. While complex, understanding the resulting ecology of influenza and its various hosts is a critical part of predicting, preparing for, and responding to potential emerging disease, whether human or animal. I will discuss our foray into the disease ecology of influenza in seals through a review of the history of influenza infection documented in marine mammals and our current efforts to track virus in the expanding population of Grey seals in the Northwest Atlantic Ocean. Following a 2011 outbreak of mortality in harbour seals in New England that was traced to an H3N8 influenza virus; we have documented annual infection in co-habitating grey seal populations, where serological evidence points to both mammalian and avian routes of exposure. Our work in seals raises questions for how ecological change and human interactions may alter the present ecology. Similar systems under investigation as well as a critical look at avian and human influenza argue for a more inclusive understanding and research approach to both the ecology of influenza and the assessment of epidemic risk. Ideally, this approach should be broad reaching and systematic.

SESSION II: SURVEILLANCE AND DISEASE INVESTIGATION

Investigating the epidemiology of swine-to-human influenza A virus transmission at agricultural colleges

Andrew Bowman, *Ohio State University, USA*

Zoonotic transmission of influenza A virus (IAV) between pigs and people at agricultural fairs is an on-going problem for both agriculture and public health. Agricultural fairs create an environment conducive to zoonotic IAV transmission by comingling pigs and people for a prolonged period of time, resulting in an increased number of variant IAV cases in people during 2011-2017. The vast majority of people who have become infected with variant IAV contracted the virus from swine at agricultural fairs, with 65 human cases being reported in association with swine at agricultural fairs during the 2017 show season alone. Research conducted by this study team provided molecular confirmation of zoonotic H3N2v transmission at county fairs and evidence that IAV infections are still common among apparently healthy swine at agricultural fairs. Surveillance efforts in 2017 found IAV infected pigs at 35 of 103 (34%) participating fairs. IAV was molecularly detected in 20% of 3150 pigs tested in 2017, with 49% of those yielding isolates. Exhibition swine are shown at agricultural fairs, but they also may be shown multiple times at various locations at exhibitions that are open to all competitors (also known as 'jackpot shows'). The vast majority of previous IAV surveillance in exhibition swine has focused on agricultural fairs and state-level jackpot shows occurring in the spring and summer, but little surveillance has occurred in the fall

and winter, creating a major gap in understanding of IAV dynamics in exhibition swine. Therefore, IAV surveillance was conducted to estimate IAV prevalence in swine at jackpot shows. Nasal wipe samples were collected from pigs at 23 jackpot shows across six states during May through July 2016 for a total of 3,754 samples. IAV was detected via RT-PCR in 461 (12.3%) samples, and viable virus was recovered from 120 (3.2%) samples. Compared to the 2016 agricultural fair season, PCR positive 19.4% and virus isolation positive 12.3%, jackpot shows have significantly lower IAV prevalence than fairs ($p<0.001$), possibly due to differences in show structure including a shorter period of pig interaction. However, progenitor viruses to the zoonotic IAVs at agricultural fairs were detected in swine at jackpot, indicating jackpot shows play a critical role in the transmission of IAVs in exhibition pigs. IAV surveillance in jackpot shows could allow early detection of IAV strains potentially threatening public health and allow for intervention prior to the summer agricultural fair season.

Isolation and characterization of a novel influenza A virus from Rousettus Aegypticus bats

Ghazi Kayali, *Human Link, Lebanon*

Bats are reservoirs for a wide range of zoonotic viruses, such as rabies, Ebola and Marburg, Hendra, Nipah, and SARS viruses. Recently, 2 influenza A/H17N10 and H18N11 viruses were detected in the little yellow-shouldered bat, in Guatemala, and the flat-faced fruit eating bat, in Peru, respectively. Since evidence showed that bats may be reservoir for novel IAV, we conducted surveillance among four bat species in Egypt. Out of 1202 swabs, 105 samples were positive for influenza A by RT-PCR. The virus was successfully isolated in eggs and purified in MDCK cells in the presence of TPCK treated trypsin. No plaques were formed in the absence of TPCK-trypsin indicating the low pathogenicity of the newly detected bat influenza virus. The purified A/bat/Egypt/381OP/2017 virus was subjected to full genome sequencing. Analysis of influenza A HA gene suggested that the A/bat/Egypt/381/2017 HA is more closely related to the Group 1 HAs rather than to the Group 2 HAs with only 70% similarity with known influenza A viruses. The NA of A/bat/Egypt/381OP/2017 had 72% amino acid sequence identity to NA of a North American H3N2 virus. Similarly, the internal genes showed no more than about 80% similarity with known viruses. The isolated virus had more affinity to avian-like sialic acid receptors. Sera raised against showed low-level cross-reactivity with H9N2 viruses and none with other influenza A subtypes. This data indicates that a novel A/H19N12 virus circulates amongst Egyptian fruit bats. This virus is more similar to avian influenza viruses and maybe related to H9N2 viruses. Further characterization of this virus is necessary to understand its virological features. Further surveillance for influenza A viruses in bats is required.

Development/update of equine influenza vaccines: a summary of some of the clinical studies and requirements for registration (and some unexpected events)

Romain Paillot[1,2,3] and Fernando Montesso[3]

[1] *LABÉO Frank Duncombe, France;* [2] *Université Caen Normandie (UniCaen), France;* [3] *Animal Health Trust, UK.*

Equine Influenza (EI) is an important respiratory disease of horses caused, at the current time, by H3N8 equine influenza viruses (EIV). Vaccination is a key strategy to prevent or control this disease, both in endemic or emergency situations. However, due to continuous antigenic drift of circulating EIV strains, new or updated EI vaccines need to be commercially available worldwide. The registration of a new or modified EI vaccine requires an accurate evaluation of their immunogenicity and efficacy through multiple clinical trials conducted in the natural host. The nature and number of clinical trials required is variable, depending of the proposed changes in terms of design and/or composition (e.g. EIV strain update of an existing EI vaccine) or the specificities and recommendations that will be reported on the vaccine label in the case of a new EI vaccine (e.g. minimum target age, duration of immunity etc.). This presentation will summarise the programme of clinical trials typically required for the development and registration of a new EI vaccine in the European Union. Such a programme usually involves immunogenicity studies (both experimental and field studies), several efficacy studies (at the onset of immunity (OOI) and duration of immunity (DOI)), multi-valence equivalence studies, etc. Some of the pitfalls and difficulties encountered will also be highlighted. As an example, the refinement of the experimental EI challenge model will be presented. Briefly, room nebulisation has been one of the chosen methods to challenge horses during EI vaccine studies. However, an increased heterogeneity of the clinical response and virus shedding was measured at several occasions after experimental infection of ponies with recent Florida Clade 2 (FC2) EIV isolates, when compared with older FC2 EIV strains. Such increased heterogeneity of the disease markers could have a significant impact on the statistical power of studies, with potential consequences in terms of study design, feasibility and cost. To counteract this problem, individual nebulisation was recently evaluated as a model refinement in order to prevent an increase of the number of animals per group (one possible solution to mitigate the increased responses' heterogeneity). Results of this approach, as part of a retrospective comparison and meta-analysis of 9 independent EIV infection studies in the natural host, will be presented and discussed.

Keynote 2: Mechanisms and consequences of influenza A virus host switching

Wendy Barclay, *Imperial College London, UK*

Influenza A viruses that infect mammals all originated in wild waterfowl. To become endemic in a novel species the virus must acquire mutations that adapt it to the new host. Adaptation of avian influenza to humans is restricted by several host range barriers. These include the requirement for efficient replication in the human upper respiratory tract and the ability to survive airborne transmission.

We recently discovered a host factor, ANP32A that is co-opted by influenza virus polymerase to support its function in the nucleus. ANP32A differs between birds and mammals and this explains the requirement for adaptive mutations in the PB2 subunit of the AIV polymerase before efficient replication can proceed in human cells.

Polymerase adaptation to human ANP32A can be achieved by single mutations in PB2, or by the acquisition of an already adapted polymerase complex during reassortment. It is possible that these two routes lead to emerging viruses with very different phenotypes and affect the severity of the pandemic virus.

The final step for human adaptation is to achieve airborne transmission. It is now evident that two quite different types of mutations in the HA protein are needed; first to adapt virus to the sialic acid receptors that predominate in human URT, and second to maintain the protein's stability in the respiratory droplets that carry the virus to and from the mildly acidic human respiratory mucosa. Viruses with unstable HA do not survive in droplets produced during nebulization or exhaled from infected ferrets. Understanding the importance of HA stability for human adaptation may aid in surveillance for high risk animal strains and in addition may improve the efficacy of live attenuated influenza vaccines especially those aimed at protecting against pandemic influenza viruses.

SESSION IV: EMERGING ISSUES AND NEW DEVELOPMENTS

Surveillance and pathogenesis of the recently identified influenza D virus

Mariette Ducatez, *École Nationale Vétérinaire de Toulouse, France*

Influenza virus D (IDV) has been discovered in swine in the USA in 2011 but has now been identified in America, Europe, Asia, and Africa. In addition to its wide geographical distribution, IDV seems to be able to infect many different mammals as IDV or antibodies against IDV have now been detected in cattle, small ruminants, swine, horses, and likely camelids, harbouring thus a very wide host tropism. We will go through the recent discoveries and discuss surveillance gaps and possible interactions between IDV and co-infecting pathogens. Pathogenesis and transmission of IDV have recently been studied in calves, giving insights on the virus tissue tropism and possible routes of infection.

Evolution of Swine Influenza Viruses in China

Huachen Zhu[1,2,3], Ziying Jin[1,2,3], Junfei Jiang[1,2,3], Xiaohui Fan[4], Malik Peiris[1], Yi Guan[1,2,3,4]

[1] *School of Public Health, The University of Hong Kong (HKU);* [2] *Joint Institute of Virology (STU-HKU), Shantou University (STU);* [3] *State Key Laboratory of Emerging Infectious Diseases (HKU Shenzhen Base), Shenzhen Third People's Hospital;* [4] *Department of microbiology, Guangxi Medical University, China*

The emergence of 2009 pandemic H1N1 influenza virus (pdm09) has demonstrated that pigs could independently facilitate the genesis of a pandemic strain. The complex origin of pdm09 and the repeated zoonotic infections caused by variant swine influenza viruses (SIVs) reinforce the importance of global influenza surveillance in pigs. Our on-going SIV surveillance in southern China has isolated 1,894 influenza A viruses from 118,801

swabs during 2009-2017, leading to an overall isolation rate of 1.59%. Full genomic sequence analysis of these isolates indicated that seven major SIV lineages were co-circulating in this region, generating 23 persistent genotypes that have sustained transmissions among pigs and 44 transient variants. Cross-country pig movement has introduced novel lineages and genotypes into southern China. Frequent reassortments between the diverse lineages have contributed to the expansion of genotypic diversity, from which zoonotic variants recurrently emerged. Avian-origin H3N2, H5N1 and H9N2 influenza viruses were also detected in pigs, but all were dead-end transmissions. The expanding diversity of SIVs in southern China might generate more zoonotic variants and pose great threat to public health, warranting a close monitoring of SIV activities and interspecies transmissions to pigs.

SELECTED ORAL PRESENTATIONS

SESSION I – VIRUS TRANSMISSION AND CONTROL

O1. Assessment of zoonotic transmission of swine influenza A viruses to naive or vaccinated ferrets

Sharon M. Brookes[1], Helen E. Everett[1], Pauline M. van Diemen[1], Alexander M.P. Byrne[1], Andrew Ramsay, Samantha Watson[1], Alejandro Nunez[2], Ana Moreno[2], Chiara Chiapponi[2], Emanuela Foni[2], Ian H. Brown[1].

[1]*Virology, Animals UK Sciences Unit and Pathology Departments, Animal and Plant Health Agency (APHA), Addlestone, UK;* [2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy*

An in vivo study was conducted to assess the infection dynamics of two swine-origin H1N1 influenza A viruses, specifically, a swine influenza A pandemic 2009 (pdm09) virus strain and human influenza A virus isolate (A/Pavia/65/16)[1] that is phylogenetically indistinguishable from avian-like Eurasian swine influenza A lineage viruses currently circulating amongst pigs in Italy. As swine influenza A viruses exhibit greater genetic diversity than influenza A viruses circulating in the human population, we also assessed whether the human 2016-17 seasonal influenza vaccine could afford protection against the two swine influenza A virus strains, using ferrets as a human model. This vaccine incorporates one H1N1 antigen, A/California/07/09 that is representative of human pdm09 viruses.

The study design used two groups of five pigs, with each group housed in separate rooms and infected with $2.4x10^7 TCID_{50}$ of one strain. The infected pigs were co-housed with a group of five naive ferrets and a group of five vaccinated ferrets held in separate cages. Both virus strains readily infected pigs and produced mild, pathogenesis profiles. Analysis of pig nasal swabs showed that both virus strains reached peak shedding levels at 2-4dpi and shedding ceased by 7dpi. Daily ferret nasal wash samples were analysed to assess potential zoonotic transmission of virus. All ferret groups, except the group that had been vaccinated and exposed to the swine-origin pdm09 virus, had a viral shedding profile in nasal wash samples characteristic of infection. Seroconversion produced antibodies that detected the pdm09 and avian-like Eurasian swine influenza A virus lineages. In contrast, the ferret group that had been vaccinated and exposed to the swine-origin pdm09 virus showed a significant reduction in viral shedding in nasal secretions. An increased influenza-specific antibody response was not detected following infection, perhaps indicating a lack of productive infection because of immunity afforded by the vaccine. All infected ferrets exhibited mild clinical signs and controlled the infection. This study confirms that vaccine and challenge strains must be highly matched in order to afford protection and also indicates that pre-existing immunity to pdm09 strains may not provide protective immunity to all currently circulating swine influenza A virus H1N1 strains. The strain that had been associated with human clinical disease was found in this study to produce mild clinical signs in pigs, the natural host and in ferrets, representing a human model. There was no evidence of increased risk in comparison to the swine-origin pdm09 strain assessed in parallel.

[1]*Rovida F et al.* (2017) Euro Surveill 22:30456

O2. Differential immunogenicity of various heterologous prime-boost vaccine regimens using swine and human H3N2 influenza viruses in pigs

Sharon Chepkwony, Elien Vandoorn, Anna Parys, Eric Cox, Kristien Van Reeth.

Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium

Heterologous prime-boost vaccination with antigenically distinct viruses within a given influenza virus subtype has been shown to result in broadly cross-reactive antibodies in poultry, humans, mouse and ferret models. We have previously performed heterologous prime-boost vaccination experiments in pigs with adjuvanted whole inactivated vaccines based on H3N2 swine influenza viruses of the European and the cluster IV North American lineages. These two viruses share only 81.5% amino acid (aa) homology in the haemagglutinin 1 (HA1). The resulting antibodies cross-reacted with over 70% of a total of 15 antigenically distinct swine and human H3N2 viruses as compared to 20% in the homologous prime-boost groups. However, they failed to cross-react with the most recent human strains. Here, we aimed to (a) compare the outcome of heterologous prime-boost vaccination with various combinations of antigenically distinct H3N2 viruses (b) examine whether it is possible to induce a pan-H3N2 antibody response against all H3N2 viruses of swine and humans. To this purpose, we vaccinated pigs with a swine H3N2 virus followed by a distantly related human virus, or vice versa, or with two antigenically distinct human viruses. A total of 55 conventional influenza native pigs were used. We prepared five UV-inactivated monovalent whole virus vaccines based on antigenically distinct swine and human H3N2 viruses. The vaccines were administered intramuscularly in combination with an oil-in-water adjuvant, 4 weeks apart. Eight different heterologous prime-boost groups were compared with five homologous prime-boost control groups and a mock-vaccinated group. The vaccine viruses shared between 79 and 84% aa homology in the HA1. Sera collected 14 days after the second vaccination were tested in a haemagglutination inhibition assay against 17 H3N2 viruses of humans and swine. The homologous prime-boost vaccinations resulted in cross-reactivity ranging from 24-47%, with antibodies in most groups mainly reacting with the vaccine and closely related strains. Heterologous prime-boost vaccinations on the other hand resulted in varying antibody cross-reactivity ranging from 0-59%. While none of the heterologous prime-boost combinations induced a pan-H3N2 antibody response, some combinations resulted in significantly broader antibody responses as compared to others. We are currently investigating the underlying reasons for these observations.

O3. Neutralizing antibody response to booster/priming immunization with new equine influenza vaccine in Japan

Takashi Yamanaka, *Equine Research Institute, Japan Racing Association*, Japan

Equine influenza (EI) vaccine has been widely used. However, the causative EI virus (H3N8) undergoes continuous antigenic drift, and the vaccine strains must be periodically reviewed and if necessary, updated to maintain vaccine efficacy against circulating viruses. In 2016, the Japanese inactivated whole vaccine was updated by replacing the old viruses with the Florida sub-lineage Clade (Fc) 2 virus, A/equine/Yokohama/aq13/2010 (Y10). In turn, the Japanese vaccine became bivalent containing A/equine/Ibaraki/1/2007 (Fc1) and Y10 (Fc2), complying with World Organization of Animal Health (OIE)'s recommendation. Here, we assessed the virus neutralization (VN) antibody response to Fc2 viruses currently circulating in Europe, after booster or primary immunization with the new vaccine. These European viruses have the

amino acid substitution A144V or I179V of the haemagglutinin protein.
In horses that had previously received a primary course and bi-annual boosters with the old vaccine, booster immunization with the updated vaccine increased the protection against the European Fc2 viruses as well as Y10. There were no significant differences in the VN titers against Y10 and the Fc2 viruses with A144V or I179V in horses that had received a primary course of the updated vaccine. However, a mixed primary course where the first dose was the old vaccine and the second dose was the updated vaccine, reduced VN titers against the European viruses compared to that against Y10, maybe due to the original antigenic sin. Because of the longer shelf life of EI vaccines (2 or 3 years) than that of human seasonal influenza vaccines (1 year), there is often an overlap of vaccines on the market and in the stores in veterinary hospitals when an updated vaccine is released. Our data suggest that the new vaccines should be used for both vaccinations when administering the primary course.
In summary, the new vaccine affords horses protective level of VN titers against the Fc2 viruses carrying A144V or I179V, but our results suggest that the combination of the old and new vaccines for primary immunization would not be optimum.
We would like to thank Dr Debra Elton (Animal Health Trust, UK) for the provision of AY13 and DV11. We also wish to thank Dr Tetsuya Nakao (Animal Quarantine Service, Japan) for the provision of Y10. Finally, we wish to thank all veterinarians belonging to the Japan Racing Association and Dr Daisuke Miyakoshi (Hidaka Horse Breeders' Association) for collecting the horse serum samples.

O4. A reassortant swine influenza A virus incorporating genes from pandemic (H1N1) 2009 and swine subtype H1N2 viruses is capable of interspecies transmission in pigs and ferrets

Helen E. Everett, Bethany Whitehouse, Brandon Z. Londt, Michael J. Kelly, Vivien Coward, Pauline M. van Diemen, Alejandro Nunez*, Ian H. Brown and Sharon M. Brookes

Virology and Pathology Departments, Animal and Plant Health Agency (APHA), Addlestone, UK*

Since the emergence of pandemic (H1N1) 2009 (pdm09) influenza A virus, this strain has become established into the UK swine population and currently co-circulates with the former endemic swine influenza A virus (SwIV) strains, avian-like H1N1 and traditional human-like H1N21. In 2010, a reassortant SwIV virus, H1N2r, which caused mild clinical disease in pigs in the UK, was isolated[2]. This reassortant virus incorporates the internal gene cassette (TRIG) from the pdm09 strain together with the genes encoding HA and NA from swine H1N2 subtypes. The potential phenotypic changes associated with a novel combination of SwIV gene segments were unknown. Therefore, we investigated the pathogenesis and infection dynamics of an H1N2r isolate in pigs, the natural host and in ferrets, representing a human model of infection.
Our results revealed that both pigs and ferrets supported productive infection with the H1N2r virus, although necroscopy analysis indicated that this virus could be more adapted to a swine host. Clinical signs and virological parameters indicated mild disease in both species. However, in contrast to pigs, ferrets shed a larger total quantity of virus over an extended period of time. Seroconversion occurred in all ferrets by 21dpi but not all pigs seroconverted. Intra-species transmission by direct contact could be demonstrated in both pigs and ferrets. Inter-species transmission by indirect bioaerosol also occurred, whether pigs or ferrets were the infection source. Transmission of virus from pigs occurred more rapidly in comparison to transmission from ferrets, which occurred with a lag phase of 8 days. Once animals became infected, whether directly or

indirectly, the shedding profile was comparable within the same species. These results indicate that, although this novel H1N2r reassortant virus causes mild clinical signs, it has both zoonotic and reverse zoonotic potential. Given the emergence of reassortant viruses incorporating TRIG gene segments from pdm09 strains[1], our findings have animal welfare and economic implications for the agricultural sector, as well as public health significance because of the potential for virus transmission at the human-animal interface.

[1]Watson SJ et al. (2015) J Virol 89:9920-9931; [2]Howard WA et al. (2011) EID 17:1049-1052.

O5. Antibody landscaping in the context of vaccination against influenza A viruses in European swine

Sara Lopes, Divya Venkatesh, Helen Everett, Holly Everest, James Seekings, Natalie McGinn, Steve Essen, Susan Collins, Debra M. Elton, Nicola S. Lewis

Department of Zoology, University of Cambridge, UK

Swine influenza A virus (swIAVs) presents a substantial disease burden for pig populations worldwide and poses a potential pandemic threat to humans. Controlling swIAVs in swine populations is extremely complex due to: co-circulation of multiple influenza lineages that are endemic in swine populations; differing immunity between age groups within herds and between different geographical regions; differences in vaccination programs around the world; and asymptomatic infections in swine allow viral dissemination. Vaccination strategies vary by region, with some countries having no swine vaccine programme. In Europe, commercially-manufactured multi-strain vaccines are available including a bivalent split virus product in an oil-in-water adjuvant. Improved control of influenza in the swine herd would reduce the risk of incursion into the human population. Key to any improved control measures is characterizing the swine immunological profiles to influenza infection, and establishing the relative cross-protection afforded in realistic situations on a by-animal and by-group basis.

Antibody landscaping is a technique that aims to quantitatively analyse the antibody mediated immunity to antigenically variable pathogens, achieved by accounting for antigenic variation among strains. Using antigenic cartography and antibody landscaping, here we assess the antibody mediated immunity of sera derived from partially-immune pigs (derived from vaccination) that are then challenged with a heterologous virus. Sera was collected at two different time points (V1 and V2) allowing for assessment of the duration of the immune response and characterisation any possible changes in swine antibody profile to a range of influenza viruses. We also compare individual animal responses and determine the rate and quality of cross-protection as antibody response declines over time. Using the transmission experiment results, we also determine the antigenic distance away from the vaccine strain that most effectively results in transmission to another partially-immune animal.

O6. Novel determinants of equine influenza virus morphology

Ilaria M. Piras, Daniel Goldfarb, Veronica Patton, Colin Parrish, Edward C. Hutchinson, **Pablo R. Murcia**

MRC-Centre for Virus Research, University of Glasgow, UK

Influenza A viruses (IAV) from clinical isolates are generally observed as pleomorphic populations containing both filamentous and spherical particles, while laboratory adapted

strains are generally exclusively spherical. The role that distinct morphologies play in infectivity, virulence, and transmission is unclear, and although mutations associated with each morphology have been mapped, our understanding of the genetic determinants of IAV morphology is still incomplete. **Objectives and Methods:** A/Equine/South Africa/4/2003 (SA/03) and A/Equine/Ohio/1/2003 (O/03) are very closely related equine influenza viruses (EIVs), 99% identical at the amino acid level across the entire genome. They are included as reference strains for the Florida 1 clade by the OIE Expert Surveillance Panel on Equine Influenza Vaccine composition. In this study, we observed that SA/03 and O/03 display a marked difference in morphology. Notably, this difference occurred despite the viruses encoding identical M1 proteins, which until now were considered to be the sole determinant of EIV morphology (Elton et al., 2013). To identify the molecular determinants of the morphological differences between SA/03 and O/03, we combined reverse genetics, confocal and electron microscopy. **Results:** We found that O/03 reproduces as homogeneous populations of short particles, while SA/03 virions reproduce to form a pleomorphic population similar to that of most EIVs, including microns-long filamentous particles, By using reassortant viruses generated by reverse genetics we identified genomic segments 1 (PB2), 4 (HA) and 6 (NA) as novel determinants of EIV particle shape. **Conclusions:** We identified previously uncharacterised segments as determinants of EIV morphology. As EIV morphology had been previously associated with mutations in segment 7 (M), our findings expand the repertoire of genomic determinants responsible for virion shape, including segments 1, 4, and 6. To the best of our knowledge, this is the first report of PB2 involvement in IAV morphology, suggesting that unappreciated mechanisms influence virus assembly.

O7. Impact of HA stability on influenza virus replication and transmission in swine

Charles J. Russell, Guohua Yang, Marion Russier, Peter Vogel, Richard Webby

St. Jude Children's Research Hospital, Memphis, USA

The stability of the haemagglutinin (HA) protein, or its pH of irreversible activation, has been associated with influenza A virus host range. Previous studies have suggested that avian hosts may prefer a relatively unstable HA protein (activation pH of approximately 5.5 to 6.2), while airborne transmissibility in ferrets may require a more stable HA (activation pH of approximately 5.0 to 5.5). Swine permit replication of both avian-preferred and human-preferred HA receptor binding specificity. A preference in swine for stable or unstable HA proteins was unknown, but was expected to contribute to the ability of swine to serve as an intermediate host between avian species and humans. The **Objective** of our study was to determine the range of allowable HA stability for replication and transmission of H1N1 and H3N2 influenza A viruses in swine. **Methods**: We measured HA activation pH values of circulating swine H1N1, H1N2, and H3N2 isolates. H1N1 values ranged from 5.1 to 6.0, H1N2 ranged from 5.5 to 5.9, and H3N2 ranged from 5.3 to 5.8. We generated three H1N1 viruses that ranged in HA activation pH (Y17H=6.0, WT=5.5, R106K=5.3). **Results:** Stabilized R106K replicated, caused disease, and transmitted to other swine with similar kinetics to WT virus. Both WT and R106K viruses retained parental stability and genotype after replication and transmission in swine. The destabilized Y17H virus had delayed replication and transmission in swine, caused less pathology, and evolved variants with average HA stabilities of pH 5.8 (compared to 6.0 of the input virus). All three viruses yielded airborne transmission from

swine to ferrets in adjacent cages, although transmission of Y17H to ferrets was associated with HA stabilization to pH 5.5-5.6. **Conclusions:** Overall, we found that swine tolerate a broad range of HA stability for replication and transmission that overlaps with apparent ranges of allowable HA stability in avian and ferret hosts. The data suggest replication of zoonotic influenza A viruses in swine may provide an ideal host within which avian-like influenza viruses may be able to adapt both receptor-binding specificity and HA stability to acquire transmissibility in ferret and human hosts. Our findings underscore the importance of surveillance efforts for swine influenza viruses.

O8. Dynamics and parameters of influenza A virus transmission in nursery pigs

Montse Torremorell, Fabian Orlando Chamba Pardo, Spencer Wayne, Andres Perez, Marie Culhane, Matthew Allerson

College of Veterinary Medicine, University of Minnesota, USA

Piglets at weaning play an important role in maintaining and transmitting influenza A virus (IAV) into wean-to-finish farms. Understanding IAV infection dynamics in growing pigs is critical to guide influenza control strategies in breeding herds. In this study, we assessed patterns of influenza transmission and measured influenza transmission rates, influenza clinical signs of sneezing and coughing, and average daily weight gain in 10 different cohorts of nursery pigs. We identified 4 patterns of influenza transmission based on their IAV prevalence at weaning. The first pattern had high IAV prevalence (97%-100%) at weaning with a subsequent rapid decline of infection. The second pattern had also high IAV prevalence at weaning, a rapid decline of infection, and a recurrent infection (infection and transmission of the same virus to pigs subsequent to the initial detection of the virus) four weeks later. The third pattern had I AV prevalence levels at weaning ranging between 7% and 57% with 2 peaks of infection afterwards, 1 week after weaning and 4-5 weeks later. In the fourth pattern, there were no IAV positive pigs at weaning although few pigs tested positive at different weeks after weaning. Reproductive ratios (R), which were calculated for transmission patterns 2 and 3, ranged from 3.4 to 30.8. There were no statistically significant associations between those transmission patterns and percentage of sneezing and coughing pigs or average daily weight gain. Different groups of nursery pigs showed distinct patterns of transmission and transmission parameters after weaning that likely reflect common field situations and will help us guide interventions in the breeding herds.

SESSION II: SURVEILLANCE AND DISEASE INVESTIGATION

O9. Antigenic evolution of global swine influenza A viruses and pandemic risk

Divya Venkatesh, Gaelle Simon, Severine Herve, Kristien Van Reeth, Emanuela Foni, Maria Serena Beato, Alice Fusaro, Richard Ellis, James Seekings, Susan Collins, Holly Everest, Natalie McGinn, Steve Essen, Sharon M. Brookes, Ian H. Brown, Gustavo del Real, Amy L. Vincent, Nicola S. Lewis

University of Cambridge, Cambridge, UK

Swine influenza presents a substantial disease burden for pig populations worldwide and poses a potential pandemic threat to humans. H1N1, H1N2 and H3N2 are the main subtypes of swine influenza A viruses (IAV). There have been several instances of swine

IAV transmission into humans and causing pandemics, as well and reverse movement of human IAV into swine. For example, four introductions of H3 viruses into pigs have been identified, along with two additional lineages introduced into humans from swine.
The focus of our study is the virus surface glycoprotein HA, which is the primary component of influenza vaccines, and a major driver of influenza evolution and host immune response. Antigenic cartography based on mapping haemagglutinin-inhibition (HI) assay titres has revealed a considerable diversity in global swine IAV HAs and here we present an updated account from currently circulating strains. Studies have shown that certain amino acid substitutions have greater effects on antigenicity because of their proximity to sites of functional importance, e.g. receptor binding. We find that such previously defined substitutions in swine H3 and human H3 and H1 lineages do account for some of the diversity seen in swine IAV strains. In contrast to human H3 IAV, we find no clear temporal clustering of global swine IAV HAs, and no clusters defined by a single or recognisable set of mutations(s). The genetic background on which the mutations occur appears to be an important factor in determining their effects on antigenicity of HA, and we present a lineage-wise breakdown of antigenic evolution.
We are also using cartography from HI assays with swine and ferret antisera to determine antigenic relatedness between swine and human strains to identify highly divergent swine strains. At the same time, we assess the human population immunity to currently circulating and emergent swine viruses. From experimental and field examples, we define the antigenic drift distance that is likely significant for loss of immunological cross-protection to H1 and H3 IAV in the pig.

O10. Pathogenesis and transmission of influenza A viruses with dominant H1 genome constellations found in US swine herds

Carine Kunzler Souza[1], Tavis K. Anderson[1], Bryan S. Kaplan[1], Phillip C. Gauger[2], Eugenio J. Abente[1], Amy L. Vincent[1]

[1]*National Animal Disease Center, USDA-ARS, USA;* [2]*Iowa State University, USA*

Influenza A viruses (IAV) are an economic and health burden to the swine industry and have a major public health importance. There is substantial IAV genetic diversity in pigs in the US, including distinct H1 phylogenetic clades such as 1A.1 (alpha), 1A.2 (beta), 1B.2.2.1 (delta-1a), 1B.2.2.2 (delta-1b), 1B.2.1 (delta-2), 1A.3.3.2 (pandemic) and 1A.3.3.3 (gamma). Detections of reassorted IAV in swine remains high after repeated introductions of H1N1pdm09 from humans into pig populations. A previous study identified dynamic patterns among whole-genome constellations of H1N1 and H1N2 in US pig herds from 2009 to 2016. The dominant gene constellation patterns were assigned as H1 clade, NA lineage (classical, 1998 or 2002) and internal gene lineage (TRIG - T or Pandemic - P) in the order of PB2, PB1, PA, NP, M and NS. This work demonstrated that the most abundant H1 genome patterns were gamma/N1-TTPPPT, delta-1a/N2-2002-TTTTPT, delta-1b/N2-2002-TTTPPT, delta-2/N2-1998-TTTTPT and alpha/N2-2002-TTPTPT. To understand the clinical outcome of viruses with these dominant genome constellations, we assessed the pathogenesis and transmission of isolates representing these 5 IAV genome patterns in an in vivo swine study. The gamma/N1-TTPPPT demonstrated a significantly higher percentage of lung lesions compared to the negative control and delta-1b/N2-2002-TTTPPT groups. All primary infected pigs shed virus between 1 to 5 days post infection, with subtle differences between group mean titers on different days. All viruses replicated to high titers in the lungs, and all pigs in the contact groups had detectable virus titers by 5 days post contact. Although the gamma/N1-TTPPPT presented with higher percentages of lung

lesions, shedding and transmission were comparable to the other viruses. These results indicate that viruses with these dominant HA/NA clades and genome constellations were virulent and replicated efficiently, despite containing different internal gene patterns. Additionally, further investigation of the gamma/N1-TTPPPT may indicate gene segments or mutations involved in determinants of pathogenesis/virulence in swine. Comparison of these genotypes to viruses containing genome constellations that are persistently, but less frequently, detected or viruses with genome constellations that are decreasing in detection frequency may provide a better understanding of the role genome constellation plays in the clinical/infectious phenotype of IAV in swine.

O11. Triple reassortant H3N2 with seasonal human H3, pandemic internal genes and N2 of swine origin circulates in Danish swine herds

Lars E Larsen[1], Jesper S. Krog[1], Malene R. Andersen[1], John Franks[2], Michael A. Larsen[3], Charlotte K. Hjulsager[1], Richard Webby[2]

[1]*National Veterinary Institute, Technical University of Denmark, Denmark;* [2]*Division of Virology, St. Jude Children's Research Hospital, USA;* [3]*Ceva Animal Health, Denmark*

In 2014, a Danish swine herd with confirmed swine influenza virus diagnosis had persistent problems with respiratory disease in pigs and reproductive problems in sows, despite vaccination against swine influenza. Subtyping by sequencing of the influenza virus revealed that it contained an HA that was most closely related to human seasonal influenza from 2004/05 and an NA closely related to contemporary swine N2 influenza A viruses. These findings led to further investigation of the occurrence of the new virus strain denoted H3huN2sw, in Danish swine herds. An H3hu-specific real-time RT-PCR was developed and used for screening of samples submitted to the National Veterinary Institute for SIV diagnostic purposes and the H3huN2sw virus was found in several farms in Denmark. Most samples contained H3 of 2004/5 origin, but more recent H3 introductions were also revealed. All positive samples were inoculated on MDCK cells and if no growth were observed, the sample underwent serial passage in embryonated chicken eggs. Haemagglutination inhibition test against hyper immune sera of pigs vaccinated with a commercial swine influenza vaccine available in Denmark was performed to investigate vaccine effectiveness. The zoonotic potential of this novel virus was assessed in the ferret transmission model.

Three ferrets were inoculated with an H3huN2sw isolate and housed in separate cages, each together with a naive ferret to study direct transmission. In adjacent cages were three additional ferrets housed to study aerosol transmission. HI testing revealed that there was no cross-reactivity of the vaccine antisera to the H3huN2sw. The ferret experiment revealed that the virus was readily transmitted to contact animals, but that no aerosol transfer was observed. The H3huN2sw circulating in Danish herds seems to cause more pronounced disease in pigs than other circulating SIVs. Furthermore, there is no vaccine available at the moment to control the infection with devastating consequences for swine health. Moreover, the ferret studies showed that the H3huN2sw can infect ferrets by direct contact and by that may be a potential zoonotic threat.

O12. Influenza A surveillance in the pig population of Great Britain (1991-2017)

Sharon M. Brookes, Scott M. Reid, Susanna Williamson, Stephen C. Essen, Christine Russell, Ian H. Brown

Department of Virology, Animal and Plant Health Agency-Weybridge, UK

Objectives: Swine influenza is an economically important viral disease of pigs. As a reservoir of evolving viruses, it represents a continuing threat to production animals and zoonotic risk for humans. **Methods:** In Great Britain (GB), scanning surveillance for swine influenza A virus (swIAV) began in 1991 including epidemiology and virological analyses. This has recently been augmented by phylogenetic and antigenic characterisation of swIAVs in GB through participation in the European Surveillance Network for Influenza in Pigs. **Results:** Between 1991 and 2017, 3862 submissions have been tested (range 27-275 per annum), totalling 513 positive submissions resulting in approximately 635 virus isolates. Four main sub-types of swIAV have been recovered with 2-4 sub-types co-circulating: avian-like (av) H1N1 (n=232, 45.2%), H1N2 (n=73, 18.2%), H3N2 (n=29, 5.6%) and latterly pandemic H1N1 (n=77, 15%). The H3N2 sub-type has not circulated in GB since 1997. Classical swine H1N1 was also detected during the late 1980's but not after 1993. Since 2010, the number of reassortant H1N2 viruses containing an H1N2 (external gene) - pandemic H1N1 (internal gene) has expanded. The frequency of detection of avH1N1 has declined since the initial identification in 1992 (23% to <1% of submissions) and whilst the rate of detection of H1N2 peaked in 1998 (9.8%), it declined to <1% in 2007/2008 but has increased since 2009 (~5%), likely as a result of the H1N2-pandemic event(s). The pandemic H1N1 sub-type appeared in 2009, peaked in 2010 (~8%) and now comprises <5% of submissions. Demographic analysis of two subsets of swine submission data (1998-2006 and 2009-2012) has also been performed including; the frequency of swIAV submissions and virus positives per pig population, geographical distribution, seasonality, pig age, clinical signs and inter-current disease. **Conclusions**: Swine influenza surveillance is required to detect both existing and novel swIAV sub-types in GB pigs, and monitoring of prevailing disease trends. Such analyses may identify changes in the epidemiology of swIAV of relevance to public and veterinary health with respect to zoonotic and reverse zoonotic transmission, as well as to the burden of disease for the pig industry.

O13. Genetic and antigenic diversity among recently identified human-like A(H3N2) variant viruses detected in the USA

Joyce Jones, Brian Lynch, Natosha Zanders, Marisela Rodriguez, Yunho Jang, Bo Shu, Sharmi Thor, Shannon Emery, William Davis, Ji Liu, Janna' Murray, LaShondra Berman, John Barnes, Susan C. Trock, Lenee Blanton, Sonja J. Olsen, Lynnette Brammer, David E. Wentworth, Stephen Lindstrom, C. Todd Davis

Centers for Disease Control and Prevention, Atlanta, USA

Sixty-two influenza A(H3N2) variant (A(H3N2)v) virus infections were reported to the Centers for Disease Control and Prevention from eight states during 2017. The cases were predominantly from Maryland (39) and Ohio (15), with two from Michigan, and one each from six other states. Fifty-five (88.7%) of the cases were <18 years of age, and the majority of cases reported exposure to swine after attending agricultural fairs prior to onset of illness. The haemagglutinin gene segment of all but one of these viruses was

closely related to a human seasonal A(H3N2) virus circulating during 2010/2011 that was likely introduced into the swine population via reverse zoonosis. Despite the multi-state distribution of cases, gene segments of the human-like A(H3N2)v viruses were â‰¥99% identical to each other, suggesting rapid expansion of this lineage among exhibition swine. Following the detection of 16 human-like A(H3N2)v virus infect ions in the U.S. during 2016, the World Health Organization Consultation on the Composition of Influenza Vaccines selected an A/Ohio/28/2016-like virus as a candidate vaccine virus (CVV). Sequence comparison of the 2017 viruses to this CVV identified amino acid changes in the haemagglutinin at positions within a putative receptor-binding site and antigenic sites A and D. Antigenic testing of these viruses showed reduced inhibition by ferret antisera raised to the 2016 human-like A(H3N2)v viruses and the A/Ohio/28/2016-like CVV as compared to the homologous virus titers. The 2017 A(H3N2)v viruses also displayed poor reactivity to antisera raised against A/Perth/16/2009, the closest human seasonal vaccine virus. Pooled, adult post-vaccination antisera reacted with these viruses at titers that were comparable to those against human seasonal H3N2 vaccine strains; however, pooled sera collected from children had reduced titers compared with those of adults. The difference in serum titers between children and adults and the number of cases <18 years of age suggests a greater risk of infection in children due to lack of pre-existing immunity. Based on this antigenic variation, development of an A(H3N2)v CVV antigenically related to the 2017 viruses was recommended.

O14. Improved surveillance using primary respiratory epithelial cells

Stacey Schultz-Cherry, Sean Cherry and Victoria Meliopoulos

Department of Infectious Diseases, St Jude Children's Research Hospital, Memphis, USA

In spite of significant advances in molecular virology, a key goal of surveillance remains viral isolation. Yet we know that isolation can be difficult, time and resource consuming, and dependent on the use of cell lines that may lead to genetic changes in the virus during passaging. In response, we undertook a project to isolate primary respiratory nasal, tracheobronchial and alveolar cells from a variety of species, develop cell lines, and determine if these cells would increase isolation rates during surveillance. Our preliminary studies suggest that inoculation of swine nasal (sNEC) and tracheal (sTEC) primary or cell lines with swine nasal swab or oral fluids leads to increased viral isolation and higher viral titers. On-going studies are examining the impact of repeated passaging in sNEC/TEC cells as compared to MDCK on genetic stability and their use to isolate variant swine viruses from human samples.

O15. Surveillance of swine influenza A, B, C and D viruses in Europe 2015-2017

Dinah Henritzi, Silke Wacheck, Martin Beer, Timm C. Harder

Institute of Diagnostic Virology, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Germany

Influenza A virus (IAV) infections causing economic losses are widely spread among swine populations worldwide. In Europe, over the past decades, four lineages of reassortant viruses between avian and human viruses have formed (H1N1av, H1N2hu, H3N2, H1N1pdm/2009) that infect swine. In addition to type A single cases of influenza

B virus (IBV) and influenza C virus (ICV) infection were reported in pigs. Also a newly described influenza D virus (IDV) has been associated with respiratory illness in swine. The emergence of the most recent human pandemic influenza virus (H1N1pdm/2009) from reassortant porcine influenza viruses underlines the importance of swine populations as carriers of influenza lineages with zoonotic and even pandemic potential. In view of the One Health concept a closer surveillance of these populations therefore seemed a logical consequence. However, surprisingly few countries actually embarked on sustained, governmentally driven and publicly controlled monitoring programs. A passive surveillance program for SIV in pig populations in selected European countries has been initiated on basis of funding by a veterinary vaccine producer as a follow-up of the public ESNIP3 program.

The Surveillance started in April 2015 and, until end of 2017, comprised up to 18.000 samples. It targeted nasal swab samples collected from pigs showing clinically apparent respiratory disease. Samples were screened by real time RT-PCR (RT-qPCR) for presence of influenza A, B, C and D viruses (*Henritzi et al*., 2016; *Henritzi et al*., submitted). IAV positive samples were subjected to molecular subtyping, virus isolation, antigenic and phylogenetic characterization. A high incidence of IAV-infections affecting about one quarter of the pigs with clinically apparent respiratory problems and representing all four lineages and various reassortants between them was detected in a season-independent manner. Increased findings of pandemic H1N1/2009 and co-infections with different H1 subtypes were repeatedly documented as well as the occurrence of a new spill-over of a human seasonal H3-subtype into the swine population. Prevalence of the different lineages were geographically restricted and incursions of new lineages and/or reassortants were documented for several European countries. In contrast to widespread IAV infections only two IBV and one IDV case were found, indicating that Influenza B, C and D viruses do not play a major role as pathogens in swine with respiratory illness in Europe.

O16. The other influenza surface glycoprotein: Probing the antigenic differences between N2 neuraminidase lineages of North American swine influenza A viruses

Bryan S. Kaplan, Tavis K. Anderson, Jefferson Santos[2], Daniel Perez[2], Nicola Lewis, Amy L. Vincent

[1]National Animal Disease Center, USDA-ARS, USA; [2]University of Georgia, USA; [3]Animal and Plant Health Agency-Addlestone, UK

Influenza A viruses (IAV) are endemic respiratory pathogens of swine that constitute a substantial economic burden to swine producers. Vaccination is the most commonly employed control measure, though the substantial diversity of IAV circulating in the US swine population adds tremendous challenges to effective vaccine formulation. The neuraminidase (NA) protein of IAV is a surface glycoprotein, important for the release of nascent virus particles from the cell surface. Though not as protective as neutralizing antibodies against the haemagglutinin (HA), antibodies against the NA can provide protection from influenza infection and transmission, particularly when HA immunity is diminished by drift. H3N2 and H1N2 viruses widely circulate in North American swine populations, but the N2 is divided into two distinct phylogenetic lineages resulting from introductions of human H3N2 in 1998 and 2002, with further genetic diversity within the 2 lineages. Here, we assessed the antigenic differences between and among 1998 and 2002 N2 lineages of swine IAV. N2 antigens were generated by reverse genetics on an

irrelevant H9Nx backbone, with the N2 derived from viruses representing the contemporary phylogenetic diversity of N2-98 and N2-02 NA lineages. Using an enzyme-linked lectin assay (ELLA) we assessed neuraminidase inhibition (NI) titers using the H9Nx antigens and a panel of swine antisera against wild-type H3N2 or H1N2 viruses from four genetic clades within the N2-98 lineage; or the four genetic clades within the N2-02 lineage. Varying intra-lineage cross-reactivity was observed between N2-98 and N2-02 clades. Antisera raised against N2-98 antigens did not display cross-reactivity against N2-02 antigens, but antisera raised against N2-02 antigens had some cross-inhibitory activity against the limited panel of N2-98 antigens. Using antigenic cartography, we mapped the N2 antigens and calculated the antigenic distances between N2 lineages and clades. These preliminary results begin to identify the antigenic differences the two major N2 lineages circulating in North American swine populations and provide encouraging evidence for a need to expand the antigen and anti-sera panels within the predominant N2 phylogenetic clades. This will enable identification of specific residues contributing to antigenicity to improve vaccine strain selection and development.

SESSION III: CLINICAL AND EXPERIMENTAL VIROLOGY

O17. Development of an in vitro model to study bacterial infection secondary to influenza A virus

Janet M Daly[1], Marta Barba Recreo[2], Lorna Beeley[1], Sharon Egan[1]

[1] *School of Veterinary Medicine and Science, University of Nottingham, UK,* [2] *Universidad Cardenal Herrera, Spain*

Influenza A virus (IAV) is a leading cause of infectious respiratory disease in equids worldwide, and secondary bacterial pneumonia worsens the outcome. Secondary bacterial infection is also a feature of canine and human IAV infection. The aim of this study was to develop an in vitro model to study the mechanisms of synergy between IAV and opportunistic bacteria such as Streptococcus equi subspecies zooepidemicus (SEZ). After screening different cell lines, canine macrophage-like DH82α cells were chosen because the interferon response to two equine H3N8 subtype IAV strains, Kentucky/5/02 and Sussex/89, mimicked the responses observed in ponies and in ex vivo explant models. In a pilot experiment, DH82α cells were infected with SEZ either 1 or 24 hours after Kentucky/5/02 infection. A notable finding was that tumour necrosis factor-alpha mRNA expression was significantly higher at 6 hours after the secondary bacterial infection than after infection with either the virus or the bacterium alone, especially when the bacterial challenge was delivered 24 hours after viral infection ($P<0.001$). Although confirmation of their relevance is required, these preliminary results suggest that DH82α cells could provide an in vitro model to investigate the role of the innate immune response in secondary bacterial infection.

O18. Evolutionarily distinct influenza A viruses show different gene regulation profiles in equine cells

Julien Amat, Joanna Crispell, Quan Gu, Ana Da Felipe and Pablo R. Murcia

MRC University of Glasgow Centre for Virus Research, Glasgow, UK

The mechanisms that allow avian influenza A viruses (IAVs) to establish as endemic

lineages in mammals are currently unknown. As viral infections require coordinated and highly specific interactions between virus and host proteins, molecular incompatibilities between them could act as effective species barriers. We hypothesize that the process of IAV adaptation to mammals is dynamic, evolutionarily driven, and involves significant changes in virus-host interactions that would result in more effective viral replication and improved counteraction of the cellular response to infection. As H3N8 AIVs are highly prevalent in wild bird populations and have jumped into horses in at least two independent occasions, they provide unique opportunities to study IAV mammalian adaptation. To test our hypothesis, we infected an interferon-competent and permissive equine cell line (E-Derms) with three evolutionarily distinct H3N8 IAVs, A/equine/Uruguay/1/1963 (EIV/63), A/equine/Ohio/1/2003 (EIV/03), and A/ruddy shelduck/Mongolia/963V/2009 (AIV/09), and we performed transcriptome analysis at 4 and 24 hours post-infection. RNA sequencing was carried out using NextSeq Illumina technology. Significant differentially expressed genes (DEGs) were identified using EdgeR and HTseq-count algorithms, and IPA software was used to determine the canonical pathways in which DEGs were involved. Our results showed that based on mRNA level, infection with evolutionarily distinct H3N8 IAVs differently affects host gene transcription. This allowed us to define important gene subsets such as i) "influenza DEGs", which include the downregulation by all IAVs of ISG15, MX1, IFIT1 ii) "equine influenza DEGs", which include the downregulation by all EIVs of IFIT3, IFIH1, IRF9, OASL, and others, and iii) "EIV/03 specific DEGs", which include downregulation of ISG20, OAS2, IFI35, and others. Our results highlight a selective regulation trend along the evolutionary history of H3N8 IAVs in horses confirmed by i) a decreasing number of DEGs going from 1092 to 455 to 104, at 4 hours post-infection with AIV/09, EIV/63 and EIV/03 respectively, and ii) a higher fold change in the regulation of specific transcripts. Moreover, EIV adaptation to horses is highly supported by our molecular pathways analysis, which highlights a specific stepwise-downregulation of Interferon, TR EM1, and RIG-I signalling pathways. In conclusion, our findings provide an insight on general strategies employed by avian influenza viruses to establish successfully in mammals.
* Equal contribution.

O19. Evaluation of equine influenza virus neutralising antibody responses induced by vaccination using a pseudotype virus based assay

Simon Scott[1],Rebecca Kinsley[1], Romain Paillot[2,3], Stephane Pronost[3], Loïc Legrand[3], Stephanie Fougerolle[3], Janet Daly[4]

[1]Viral Pseudotype Unit, School of Pharmacy, University of Kent, UK; [2] Animal Health Trust, UK; [3]BIOTARGEN EA 7450, Université Caen Normandie, UNICAEN LABÉO, France; [4]School of Veterinary Medicine & Science, University of Nottingham, UK

To date, no routine methods are available to measure the virus neutralising (VN) antibody response induced by equine influenza virus (EIV) vaccination and/or EIV infection. In this study, we aimed to use EIV pseudotyped lentiviruses (pseudotype viruses; PVs) to measure EIV-specific VN antibody responses in the natural host. PVs used were non-replicating hybrid viruses with the disabled core of a lentivirus (expressing only a quantifiable reporter gene - luciferase) and the envelope of the study virus, the EIV haemagglutinin (HA) glycoprotein (A/equine/Richmond/1/07, Florida Clade 2 strain). PVs were generated using our in-house protocols (Scott et al., 2012; 2016). Serum samples (n=134) were taken at various time intervals from naive Welsh mountain ponies that had been immunised with monovalent Florida Clade 1 or 2 (FC1 or FC2) vaccines (at 4 and 8 weeks) and later EIV challenged with EIV in week 38. Sera

were also tested by single radial haemolysis (SRH) and haemagglutination inhibition (HI) assays. Multifactorial analyses were carried out to determine statistical significance of results. All ponies were seronegative before vaccination. Pseudotype virus neutralisation (PVN) titres for non-immunised ponies remained very low until experimental infection, then markedly increased. Animals receiving both FC1 and FC2 (high dose) vaccines exhibited strong PVN titres 4 and 8 weeks post inoculation, with lower dose groups demonstrating partial seroconversion after 4 weeks and full at 8 weeks. Comparison of antibody titres between PVN and the other assays revealed a correlation coefficient (RÂ²) of 0.7 for SRH and 0.6 for HI. The PVN test showed particular sensitivity where antibody titres from SRH and HI were of low amplitude, such as within a few weeks of vaccination or later just before challenge. PVN could therefore provide a useful tool for assessing vaccine responses in situations and at time points where standard assays show limitation. In conclusion, the PVN test efficiently measures EIV neutralisation after immunisation and/or experimental infection, and utilisation has the potential to complement information provided by traditional serological assays.

O20. The bat influenza H17N10 is neutralized by broadly-neutralizing monoclonal antibodies and its neuraminidase facilitates viral egress

Nigel Temperton[1], George Carnell[1], Keith Grehan[1], Francesca Ferrara[1], Stuart Mather[1], Eleonora Molesti[1], Simon Scott[1], Martin Schwemmle[2], Alfredo Nicosia[3], Krzysztof Lacek[4],

[1]Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent, UK; [2]Institute of Virology, Department for Medical Microbiology and Hygiene, University of Freiburg, Germany; [3]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Italy; [4]Laboratory of Virus Molecular Biology, University of Gdansk, Poland

The diversity of subtypes within Influenza A has recently expanded with the identification of H17N10 and H18N11 from bats. In order to further study the tropism and zoonotic potential of these viruses, we have produced lentiviral pseudotypes bearing H17 and N10. These pseudotypes were shown to be efficiently neutralized by the broadly neutralizing HA stalk monoclonal antibodies CR9114 and FI6. We also confirm that H17 does not use sialic acid as its cellular receptor, as pseudotypes bearing H17 HA glycoprotein are released into the cell supernatant in the absence of neuraminidase. H17 pseudotypes are also unable to transduce cells that are permissive to non-chiropteran influenza A and B pseudotypes. We demonstrate that N10 can facilitate H5 and H7 influenza pseudotype release in the absence of another source of neuraminidase. Despite this, the N10 protein shows no activity in an enzyme-linked lectin assay. This lentiviral pseudotype system will permit research on b at influenza tropism, restriction and sero-epidemiology, without the constraints or safety issues with producing replication-competent virus, to which the human population is naïve.

O21. *In vivo* evaluation of monovalent vaccines against challenge with a contemporary alpha H1N2 influenza A virus in swine

Susan E Detmer, Winter-Viana V, Harding JCS

University of Saskatchewan, Canada

The North American swine H1 alpha cluster (1A.1.1) has evolved into three distinct genetic sub clusters. The alpha-3 sub cluster has a two-amino acid deletion at H1 positions 146-147. This sub cluster has experienced rapid spread among Manitoba's

swine herds and significant spatial dissemination from the heartland of the United States to Appalachia. While most influenza A viruses of swine cause a self-resolving respiratory disease, many of the alpha-3 strains have high rates of mortality due to severe viral pneumonia, secondary bacterial infections, and overactive immune responses. Additionally, there was one zoonotic alpha-3 infection reported in the United States. Autogenous vaccines have some success (>50%) at control in Manitoba with no virus detected within 6-8 months of sow vaccination. Therefore, whole inactivated virus monovalent vaccines were developed against one alpha-1 virus (SD0154) and two alpha-3 viruses (SD 0142 and SD0191) to examine efficacy against challenge with an alpha-3 virus (SD0191). SD0154 and SD0142 were heterologous to the challenge virus having 86.1% and 96.7% amino acid similarity with 22 and 3 amino acid differences within antigenic sites, respectively. Pigs were randomly allotted to 4 treatment groups of 10 pigs each and 4 pigs to the negative control group. Pigs in the 4 treatment groups were vaccinated twice, 14 days apart. Positive control pigs were vaccinated with sterile adjuvant. Pigs were challenged with 10^5.62 TCID50/ml with 1 ml intratracheally and 0.5 ml in each nostril 21 days after second vaccination. Nasal swabs and rectal temperatures were collected for 5 days post-challenge (DPC). Serum was collected immediately prior to challenge. Lung and bronchoalveolar fluid were collected at termination (5 DPC). Lung was scored macroscopically and microscopically, and all samples were tested by matrix RT-qPCR. Virus titration was conducted on samples with Ct <32. Haemagglutination inhibition (HI) titers for pigs vaccinated with SD0142 were higher for SD0191 than the homologous virus. Cross-HI titers for several viruses and their monovalent immune sera within the alpha-3 group have had similar results, indicating a need for additional confirmation of these reactions using microneutralization assays. The vaccinated animals had significantly lower macroscopic and microscopic lesion scores compared to the positive controls. Pigs with homologous vaccine (SD0191) had the greatest protection and least viral shedding, followed by the similar heterologous virus vaccine (SD0142) and the alpha-1 virus vaccine (SD0154). These results indicate that cross-protective properties within the alpha-3 sub cluster warrant further investigation for prospective vaccine use. Bivalent and trivalent vaccine formulations will need to be assessed in future challenge studies before the creation of regional vaccines.

O22. Novel vaccines against Swine Influenza A virus

Pauline M. van Diemen, A Ramsay, V Coward, M Aramouni, L Canini, C Charreyre, IH Brown, M Woolhouse, E Tchilian, S Gilbert, SM Brookes, B Charleston, HE Everett

Animal and Plant Health Agency-Weybridge, Virology Department, UK

Swine Influenza Virus (SwIV) has a high economic burden for the pig industry as well as zoonotic potential. The 2009 pandemic H1N1 "swine origin" infection (SwIVpdm09) is now endemic in both pigs and humans. Commercial SwIV vaccines are not regularly updated, and only recently a commercial vaccine containing the SwIVpdm09 strain has been licensed. Using this vaccine pigs were shown to have reduced virus in the lungs and excretions from the nose, this partial protection only lasted for three months. Our study evaluated the host immune responses to, and efficacy of, different vaccination strategies against a SwIVpdm09 strain, and assessed transmission of this virus from vaccinated to naive pigs. Groups of pigs were prime-boost vaccinated (3 week interval) with one of the following 8 "vaccines" 1) monovalent inactivated A(H1N1)pdm09 vaccine ("homologous") in TS6 adjuvant, 2) monovalent inactivated H1avN1 vaccine ("heterologous") in TS6 adjuvant, 3) control immunised with the TS6 adjuvant and egg fluid, 4) MVA viral vectored homologous H1-homologous NP-M1 vaccine (MM1353), 5) MVA viral vectored heterologous H1av-homologous NP-M1 vaccine (MM453), 6) Adeno

(prime) and MVA (boost) vectored homologous H1-homologous NP-M1 vaccines (AM1353), 7) Adeno-MVA vectored irrelevant antigen (AMcontrol), or 8) S-Flu, pseudotyped H3N2, a broadly protective cell mediated vaccine candidate. Viral vectored regimes were included because of their potent cellular and humoral immune responses. Seven weeks after boost vaccination, pigs were challenged with A/swine/England/1353/2009 (H1N1pdm09). Two days later, naive pigs were housed with each vaccinated/challenged group. Samples were taken before and after vaccination and after challenge. Differences in the immune responses elicited by the different treatments will be assessed. Influenza A viral RNA shedding profile was determined for each pig by RRT-qPCR. All directly challenged control immunised pigs shed virus as did heterologous, MM1353, MM453, AM-control and S-Flu vaccinated pigs. Their naive contact pigs were readily infected. Pigs vaccinated with AM1353 or homologous vaccines showed minimal virus shedding at the lower limit of detection. This did not prevent 1 or 2 of the 5 contact pigs per group becoming infected after 4-5 days co-housing, respectively. Homologous vaccinated pigs had high antibody titres against H1N1pdm09 (Haemagglutination Inhibition assay), and both homologous and heterologous vaccinated pigs showed a moderate HI titre against H1avN1. S-Flu and control vaccinated animals did not produce antibodies to either strain. Current data clearly indicates that specific neutralising antibodies are required to reduce H1N1pdm09 viral replication as shown by the homologous inactivated vaccine and Adeno-MVA combination vaccinated groups encoding matched HA.

O23. Transmission of influenza A virus from nurse sows to adopted piglets during lactation

Jorge Garrido Mantilla, Marie Culhane

College of Veterinary Medicine, University of Minnesota, USA

Influenza A virus (IAV) transmission in pigs occurs mainly by direct contact with virus-laden secretions and exposure to infectious aerosols and contaminated materials. However, less is known about indirect IAV transmission in piglets during lactation. We have isolated viable IAV from the udder skin of lactating sows which suggests that skin is a suitable surface for IAV survivability and may facilitate IAV subsequent transmission. The objective of this study was to determine if IAV infection of piglets could occur after contact with IAV contaminated skin of lactating nurse sows. Two IAV-negative pregnant sows gave birth in separate farrowing rooms at the University of Minnesota experimental animal research facilities. Post-farrowing, Sow 1 and her piglets were intranasally inoculated with 10^5 TCID50/ml of A/swine/Iowa/MT_12_07_1920/2012 H1N1 IAV. Infection was confirmed in Sow 1 and her piglets with nasal swabs (NS), oropharyngeal swabs (OP) and udder skin wipes (UW) by positive rRT-PCR detection of the IAV matrix gene. Sow 2 and all her piglets remained IAV negative on NS, OP, and UW post-farrowing. In order to assess transmission from the positive nurse sow to negative piglets, positive Sow 1 was moved into a clean room and adopted all the negative piglets from Sow 2 to mimic nurse sow piglet adoption. All piglets and sows were sampled daily post-movement. All adopted piglets became IAV positive after nursing positive Sow 1. These findings confirm the IAV infection of piglets through indirect contact with infected sow udder skin. The results highlight the potential role of nurse sows on IAV transmission in swine breeding farms during the lactation period.

O24. Attachment of IDV to the respiratory tract of cattle, small ruminants, swine and horse: a call for increased surveillance

Eva Mazzetto, Zanardello Claudia, Beato Maria Serena, Schiavon Eliana, Terregino Calogero, Monne Isabella and Bonfante Francesco

Istituto Zooprofilattico Sperimentale delle Venezie, Italy

Influenza D virus (IDV) is a newly described influenza type of the *Orthomyxoviridae* family. IDV was isolated from diseased swine in 2011 and has subsequently been detected in cattle and swine, around the world. The widespread seroprevalence of IDV in cattle and its ease of replication and transmission in this species indicate that bovine may represent the natural reservoir of this virus. In addition, serological studies have identified small ruminants, horses, and humans as hosts susceptible to IDV. These findings together with experimental data proving the ability of the virus to replicate in ferrets and guinea pigs, point at IDV as a potential emerging zoonotic agent with a wide range of animal hosts. In this study, we investigated the attachment of an Italian IDV strain to the nose, soft palate, larynx, trachea, bronchi and lung tissues obtained from cattle, sheep, goat, pig and horse. A concentrated and purified IDV virus was labelled with FITC and incubated with each of the selected histological sections. Attachment of the virus was detected with a monoclonal antibody targeting the FITC molecule. Signal was amplified with a tyramide signal amplification system and the peroxidase was revealed with 3-amino-9- ethyl-carbazole. Besides the expected affinity for the upper respiratory tract of the bovine and swine species, we demonstrated that other species express the receptors requested for the attachment of IDV and could hence allow the potential replication of the virus.
The characterization of the tissue binding profile not only suggested the most suitable tissues to perform the ex vivo analyses that are currently on-going, but will eventually guide diagnosticians in the collection of the best specimens for pathological investigations, during outbreaks of respiratory infections of unknown aetiology. Through this complementary methodological approach, we could investigate basic aspects of the pathobiology and ecology of the virus uniquely relying on tissues collected at slaughterhouses, in the pursue of generating sound preliminary data to ensure the design of an in vivo study in the most fitting animal model.

O25. Mab-based competitive ELISA for the detection of antibodies against influenza D virus

Ana Moreno, Davide Lelli, Antonio Lavazza, Enrica Sozzi, Irene Zanni, Chiara Chiapponi, Emiliana Brocchi, Emanuela Foni

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy

Objectives: Influenza D virus (IDV) was first reported in 2011 in swine in Oklahoma and consequently found in cattle, sheep and goats across North America and Eurasia. Cattle have been proposed as the natural reservoir. In this study, we developed and validated a MAb-based competitive ELISA for the detection of antibodies against IDV virus (IDV-ELISA). **Methods:** Hybridomas specific to IDV were generated using Balb/C mice immunized with purified IDV/Swine/Italy/199724-3/2015. The specificity of MAbs was determined by comparing their reactivity with the homologous and other influenza A viruses along with additional bovine and swine viruses (BHV1, PI3, RSV, BVDV, PEDV,

PRRSV). IDV-ELISA was performed using the partially purified antigen coated to the plate, two serum dilutions (1/10 and 1/20) and addition of peroxidase-conjugated MAb. Results were expressed as percentage of inhibition (PI) with respect to the non-inhibited reaction. To evaluate the diagnostic performances of IDV-ELISA, we used 618 sera (204 HI test negative and 414 HI test positive) from different species: bovine (478), swine (79), wild ruminants (47), pheasant (9) and chickens (5). The agreement between IDV-ELISA and HI test was assessed by Cohen's Kappa value (K). ROC analyses were performed to enable the selection of best cut-off value and estimation of diagnostic specificity and sensitivity. **Results:** Thirty-one anti-IDV MAbs were characterized using different ELISAs, immunofluorescence and HI assays. Out of nine MAbs positive by HI one showing wide intra-type cross-reactivity was selected as competitor MAb in the IDV-ELISA. K analysis showed an almost perfect agreement (K=0.93; 95%CI -0.899-0.961) between HI test and IDV-ELISA. ROC analysis evidenced that IDV-ELISA was accurate with an Area Under Curve AUC=0.996 (95%CI 0.988 to 0.999) and high sensitivity (Se: 99.75; 95%CI 98.6 - 100.0) and specificity (Sp: 98.52; 95%CI 95.7 - 99.7). The cut-off value representing the optimal balance of Se and Sp at the first dilution 1/10 was 65% percentage of inhibition. The subsequent dilution 1/20 could be used to estimate the antibody level. **Conclusions:** These results proved excellent diagnostic performances of IDV-ELISA, which compared to HI test presented mayor advantages, such as suitability for automation, low dependence to individual skills, spectrophotometric reading and easy interpretation of the results. This assay can potentially be exploited to detect antibodies against IDV in different animal species.

O26. Isolation and characterization of equine-like H3 viruses from wild birds in Chile

Stacey Schultz-Cherry, *St Jude Children's Research Hospital, Memphis, USA*

Equine influenza H3N8 virus was first reported in 1963 during an outbreak in horses in Florida that had been recently imported from Argentina. It has been speculated that the H3N8 virus was introduced into horses from birds. However, when and where this occurred and whether birds harbour equine-like genes remain unknown. During longitudinal surveillance in central Chile, we isolated three distinct H3 viruses from wild birds that have HA and 3 internal genes more similar to equine viruses than avian. Characterization of these viruses is underway. Although this meeting is not focused on avian influenza viruses, we hope these viruses will be of interest to the equine influenza community. We will also provide more information on the equine influenza outbreak in Chile.

O27. Outbreaks of influenza of swine and human origin in mink (*Neovison vison*)

Charlotte Kristiane Hjulsager, Jesper Schak Krog, Mariann Chriél, Gitte Larsen, Lars Erik Larsen.

National Veterinary Institute, Technical University of Denmark, Denmark.

Influenza A virus infections in farmed mink, that are associated with respiratory disease, have occasionally been reported from mink producing countries. The viruses isolated have mainly been of avian or swine origin. Infections in mink with seasonal human influenza viruses have been inferred mainly from antibody detections. In 2009, the first outbreak with Influenza A virus was recognized in Danish farmed mink.

The virus was a novel reassortant H3N2 virus. The HA and NA genes were most closely related to the 2005/06 human seasonal influenza virus and the internal genes were of contemporary swine influenza virus origin. All the infected farms received feed from the same feed producer. The feed contained fresh swine offal and the outbreak was therefore suspected to be feed-borne. Since 2009, Influenza A viruses have been detected in farmed mink in Denmark almost every year. Outbreaks are typically associated with sneezing, pneumonia and haemolytic E. coli infections. Characteristic is also bleeding from the nose. The mortality varies but is normally between three to five per cent in the affected farms.
The aim of this study was to elucidate the origin of influenza A viruses detected in Danish farmed mink in recent years by genetic and phylogenetic analyses of influenza A virus genes. The results showed that the viruses involved were either closely related to contemporary swine influenza viruses (avian-like H1N2 or H1N1) or to H1N1pdm09. The 2009 H3N2 virus has not been detected since 2009. The avian-like HA swine H1N1 and H1N2 viruses have never been detected in humans in Denmark, but are the most prevalent subtypes detected in the Danish swine herds with respiratory disease. Thus feed content of swine origin is a likely source of these viruses in mink.
The H1N1pdm09 viruses have been circulating in Danish swine since 2010 and the same subtype is now considered seasonal influenza virus in humans, rather than being "pandemic". Genetic analyses showed that some of the H1N1pdm09 viruses found in mink had a higher level of identity to H1N1pdm09 strains detected in humans than in swine. This suggests that these viruses were transmitted directly from humans to mink.

O28. H7N2 feline influenza virus evaluated in a poultry model

David L. Suarez, Mary Pantin-Jackwood

Southeast Poultry Research Laboratory, United States Department of Agriculture, USA

In November and December of 2016 a novel influenza virus was isolated from cats from an animal shelter from New York City (NYC). The virus caused respiratory disease and was found in cats in several shelters in NYC, and one human also became infected. The H7N2 subtype isolate was sequenced and it was found to be closely related to avian influenza viruses that had circulated in live bird markets in the Northeast from 1994-2006. The virus was genetically closest to poultry viruses that were isolated from around 2000 and even had a unique deletion in the HA gene only seen in the H7 LBM lineage. Surveillance of the LBMs has not detected any H7N2 viruses of this lineage since 2006, and the source of the infection to cats appears unlikely to be from poultry. A chicken and duck transmission study was performed to provide additional evidence to determine if poultry had a possible role as source of the virus. The feline influenza virus was used to infect chickens at different doses and naïve contact controls were added two days later. Three H7 LBM lineage avian viruses were included in the study as well. No evidence of productive infection was seen in chickens with the cat virus, although not all chickens became infected with the avian viruses used in the same study. Eight ducks were also challenged with a high dose of the feline virus. Three ducks were positive by serology at 10 days post challenge, but only a few ducks shed low levels of virus at days 2 or 4 post-challenge. The feline viruses do not appear adapted to poultry and it seems unlikely that the H7N2 LBM lineage circulated in poultry undetected for over 10 years. The source of infection of the feline virus remains unknown.

O29. The evolution and epidemiology of H3N2 canine influenza virus in the USA and Asia

Colin R. Parrish[1], Ian E.H. Voorhees[1], Benjamin D. Dalziel, Edward J. Dubovi[1], Amy Glaser[1], Sandra Newbury[3], Pablo Murcia[5], Laura Goodman[1], Christian Leutenegger[4], Edward C. Holmes[6].

[1]*Cornell University*; [2]*Oregon State University*, [3]*University of Wisconsin*, [4]*IDEXX Laboratories*, *USA*; [5]*University of Glasgow*, *UK*; [6]*University of Sydney*, *Australia*

The avian-origin H3N2 canine influenza virus (CIV) transferred to dogs in Asia around 2005, soon becoming enzootic throughout much of China and Korea before reaching the USA by early 2015. Phylogenetic analysis of complete genome viral sequences revealed strong geographic structuring, with clear separation between Chinese and Korean viruses, and between viruses from Asia and the USA. Within the USA the first H3N2 CIV epidemic, introduced from Korea, was maintained around the Chicago area with limited outbreaks in other regions; this population contained two distinct clades of virus before and after mid-2016. A second distinct Korean-like virus was introduced into the USA in early 2017, spread through the south-eastern states, and was then detected in other regions. Notably, all of these lineages were characterized by similar numbers of non-synonymous mutations at distinct positions across the genome, some of which are likely to influence host range or antigenic variation. The basic reproductive number, R0, of CIV in the USA varied among outbreaks, with a majority of outbreaks having an R0 between 1-2, consistent with maintained, but heterogeneous circulation. Overall, the epidemiology of the virus in the USA is characterized by numerous small monophyletic outbreaks interspersed with geographically localized epidemics. In some cases, city- and regional-scale epidemics were prolonged, and occurred simultaneously in multiple cities across the US. All of the H3N2 CIVs showed similar rates of genomic sequence evolution, both after emergence and spread within dogs in Asia, as well as during the epidemics within the USA.

P1.Equine H3N8 influenza in Egypt: An extended story with multiple ends

Basem M. Ahmed[1], Shimaa Ghoneim[1], Janet M. Daly[2]

[1]*Department of Virology, Faculty of Veterinary Medicine, Cairo University, Egypt,* [2] *School of Veterinary Medicine, University of Nottingham, Nottingham, UK.*

Almost a decade after the previous outbreak in 2000, equine influenza re-emerged in Egypt in 2008. The outbreak was extensive and included almost all horses, donkeys and mules; most cases were complicated with secondary bacterial infections. With the aim of full identification and production of novel vaccines the virus was isolated and identified as Florida clade1 H3N8 virus. We continued our work by preparation of DNA plasmid expressing HA1 domain of the virus and it was immunogenic in Wistar rats by means of haemagglutination inhibition and virus neutralization tests. A recombinant bacmid harboring the full-length H3 sequence was also prepared for production of subunit vaccine candidate based on Baculovirus expression technology. Now, the virus is being prepared for direct sequencing to study the extent of egg adaptation. In the meantime, an expected outbreak of equine influenza in unvaccinated horses in Egypt is to be identified.

P2. An automated annotation tool and unified nomenclature system for H3 haemagglutinin genes from swine influenza A viruses

Tavis K. Anderson[1], Yun Zhang[2], Catherine A. Macken[2], Richard H. Scheuermann, Amy L. Vincent[1,2]

[1]*National Animal Disease Center, USDA-ARS, USA;* [2] *World Organization for Animal Health/ Food and Agriculture Organization (OIE/FAO/OFFLU) Swine Influenza Working Group*

Epidemiologic and phylogenetic analyses of influenza A viruses (IAV) provide rational criteria for vaccine strain selection, control strategies, improved diagnostic tests, and may identify strains with pandemic potential. The haemagglutinin (HA) H3 subtype has been circulating in swine since the 1968 human influenza pandemic. Over time, repeated introductions of human seasonal H3, in conjunction with primarily N2 neuraminidases, have resulted in 9 genetically distinct H3 lineages circulating in swine globally. Due to limited global data and regionally restricted circulation, the naming of these genetic clades has lacked a comprehensive framework, resulting in inconsistent regional naming conventions that do not reflect evolutionary history. We proposed phylogenetic criteria for a globally consistent nomenclature of divergent swine H3 viruses. Further, we developed and implemented an annotation tool that assigns these biologically informative lineage categories to observed sequence data. This tool classified 2070 swine H3 HA sequences collected between 1969 and 2017. To provide evolutionary context, these data were combined with 19014 HA H3 virus sequences sampled from the same time period (2701 avian, 11910 human seasonal, 3913 equine, and 490 canine). Our proposed phylogeny-based nomenclature currently designates the nine distinct co-circulating swine clades; an additional 6 decadal clade classifications for human H3-HA to

incorporate other minor swine incursions; 5 classifications for avian H3-HA clades; 2 classifications for canine H3-HA; and a single clade classification for equine H3-HA. Our annotation tool assigned swine H3 sequences to the correct clade more than 95% of the time. In addition to the nine contemporary swine H3 clades, these analyses revealed 30 additional human-to-swine transmission events over the past 50 years. However, it remains to be determined if these introductions resulted in onward transmission in the swine population since evidence from current surveillance data was lacking. Our nomenclature and classification tool for swine H3 viruses provides a robust and accurate method for researchers to assign clade designations to publicly available or privately generated HA sequences. The classification tool can be updated readily as new clades emerge, assuring its continued relevance. A common global nomenclature facilitates comprehensive comparisons of IAVs infecting mammals and birds, within and between global regions. Importantly, our global nomenclature can ultimately provide insight into the scope of the global diversity and evolutionary patterns of swine H3 influenza virus and the impact on vaccine strain selection and diagnostic capabilities.

P3. Novel European Swine Influenza genotypes identified in Italy between 2013 and 2017

Maria Serena Beato[1], A. Fusaro, G. Zamperin, A. Milani, L. Cavicchio, A. Schivo, C. Mantovani, I. Monne, D. Vio, E. Schiavon, M. Giorgiutti, A. Castellan, M. Mion.

[1]*Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Italy*

Passive surveillance of Swine Influenza virus (SIV) in north-eastern Italy (Veneto and Friuli Venetia Giulia regions) carried out between November 2013 and June 2017 identified fifteen infected farms in fifteen different municipalities covering four neighbouring provinces. *Watson et al*. (2015) had previously described 25 different SIV genotypes in Europe (A-W). To explore the genetic diversity of the circulating SIVs in north-eastern Italy, we genetically characterized the complete genome of 36 viruses (7 H1N1, 27 H1N2 and 2 H3N2) collected from all the affected farms using the Illumina MiSeq. Topology of the maximum likelihood phylogenetic tree obtained for the H1 gene showed that the viruses belonged to four distinct lineages, namely 1B.1.2.2 (human-like H1N2), 1A.3.3.2 (A(H1N1)pdm09), 1C.2 and 1C.2.1 (avian like H1N1 and H1N2), according to the classification by Anderson et al. (2016). Phylogenetic analyses of the eight gene segments identified eight different genotypes - six (A, B, D, F, P and T) previously described by Watson et al. (2015) and two (X and Y) here described for the first time. These new genotypes have been generated from the F and T genotypes through reassortment events involving the M and the NS genes, respectively. Genotypes A, D and P have been widely co-circulating in this area for at least 3 years, while the remaining genotypes have been identified in few farms and only for a short period of time. Interestingly, genotype T viruses, which have never been reported in Italy before, were detected in 2017 in two farms. These viruses cluster separately from the European viruses of the same genotype, which suggests that they may have been generated through independent reassortment events between the pandemic lineage and clade 1C.2. The many reassortment events identified in this small geographic area, as well as the high number of co-circulating genotypes (F, P, T, X and Y) containing the matrix gene of the A(H1N1)pdm09 lineage, which had previously been associated with increased transmissibility in guinea pig and ferret models, may pose a potential public health risk and therefore their persistence in pigs should be carefully monitored.

P4. Optimisation of the culture and detection methods for Influenza D viruses

Alexander MP Byrne[1], James Snowden[1], Vivien Coward[1], Chiara Chiapponi[2], Emanuela Foni[2], Helen E Everett[1]*, Sharon M Brookes[1] and Ian H Brown[1]

[1]*Virology Department, Animal and Plant Health Agency (APHA-Weybridge), UK;* [2] *Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia, Italy.*

Introduction: Since the identification and isolation of influenza D from swine in the United States in 2011, this novel virus has been detected in swine, cattle, horses, ruminants and humans in Europe, Africa, North America and Asia and has been associated with respiratory disease in some cases. However, only a limited survey has been conducted to assess the circulation of influenza D in livestock within the United Kingdom. **Methods**: Using a representative strain of influenza D from Europe, D/swine/Italy/199724-3/2015, three different cell lines were assessed for their ability to support the replication of these strains. The cell lines used were a human colorectal adenocarcinoma (Caco2) cell line, the Madin-Darby canine kidney (MDCK) cell line and the new-born pig tracheal (NPTr) cell line. The growth of these virus strains in the different cell lines was monitored using a haemagglutination assay. Serum raised against influenza D was also used to detect the virus using a haemagglutination inhibition (HI) assay. A RRT-PCR method to detect both D/swine/Italy/199724-3/2015 and D/swine/Oklahoma/1334/2011 was also developed based on previous work and the ability of several other influenza diagnostic RRT-PCR methods to detect influenza D was also assessed. **Results:** It was found that the Caco2 cell line was superior to both MDCK and NPTr cell lines for the amplification of D/swine/Italy/199724-3/2015 virus, which was actively detected by both haemagglutination and HI assays. It was also found that the Nagy (*Nagy et al. 2010*), Spackman (*Spackman et al. 2002*) and Spackman "Perfect Match" (*Slomka et al. 2010*) M-gene RRT-PCR methodologies currently used for influenza A screening in livestock were not able to detect either influenza D strains. Based on this we adapted a previously published RRT-PCR method (*Hause et al. 2011*), which was able to detect both D/swine/Italy/199724-3/2015 and D/swine/Oklahoma/1334/2011 with comparable sensitivity. **Conclusions**: By optimising the viral culture and detection methods of influenza D viruses, we are now in a position to perform a large-scale survey of swine and cattle in the United Kingdom to assess the prevalence of this novel pathogen in livestock.

P5. Whole genome characterization of Influenza D viruses detected in cattle herds in Northern Italy between 2015 and 2017

Lara Cavicchi, A. Fusaro, G. Rizzo, A. Milani, A. Salviato, G. Zamperin, M.S. Beato, E. Schiavon, L. Bano, E. Foni, I. Monne[1]

[1]*Istituto Zooprofilattico Sperimentale Delle Venezia, Italy*

Recent studies carried out in the United States of America (USA), Europe, China and Japan have identified a new viral Genus within the Orthomyxoviridae family, classified as Influenza D virus (IDV). This newly emerged virus exhibits a broad host range and is capable of infecting swine, cattle, sheep, goats, ferrets and guinea pigs. In Italy, IDV was first detected in archived samples collected between 2014 and 2015 from cattle and swine in the Po Valley area. Here we report the genetic characterization of IDV viruses detected in North of Italy, namely Veneto, Lombardy and Piedmont, through passive

surveillance programs between September 2015 and October 2017. A total of 309 cattle farms were tested for a total of 482 samples, including nasal swabs, lungs and bronchoalveolar lavage fluid. Thirty cattle herds turned out to be positive for IDV, for a total of 40 samples positive by Real Time RT-PCR targeting the PB2 gene. The highest rate of positive samples were nasal swabs. Representative IDV positive swabs were sequenced on an Illumina Miseq platform. Phylogenetic analyses were performed for each genome segment. The analyses of the seven gene segments demonstrated that the viruses identified in the North of Italy clearly grouped within a genetic cluster composed by the sequences of the IDVs previously described in Italy and in the USA, thus suggesting a common origin for these viruses. Interestingly, the IDVs identified in Italy presented a low similarity (96.1% to 98.8% for the seven gene segments) with the French IDVs, which is the only other European country where this pathogen has been identified and characterized so far. The ability of IDV to reassort, as well as the wide host range, which characterizes this virus, are a matter of concern. Results of this study indicate that the virus is extensively circulating among bovine herds in Northern Italy and highlight the need to perform IDV surveillance on an ongoing basis to track its spread and evolution.

P6. Serologic surveillance of avian H5N2 influenza A virus infections among pigs in Minnesota, USA

Marie Culhane, Thomas Bakken, Devi Patnayak

[1]*University of Minnesota College of Veterinary Medicine, USA*

Swine-to-turkey transmission of H1 and H3 influenza A viruses (IAV) has been reported in several countries. However, it is not known how often turkey-to-swine transmission occurs nor the likely routes of transmission. More importantly, with the H5N2 highly pathogenic avian influenza (HPAI) outbreak in Minnesota, USA turkeys in 2015, and the presence of H5N2 HPAI in aerosolized particles, we need to understand the potential for swine infections with HPAI H5N2. Herein we report the results of retrospective serosurveillance for H5N2 HPAI antibodies in Minnesota swine from herds co-located in geographical regions with HPAI H5N2 infected turkeys. Sera were collected from 907 mature swine from 48 different herds located in 15 Minnesota counties between August and December of 2015. Swine were six-months of age or older at the time of collection in order to include sera from swine with the potential exposure to HPAI H5N2 infected turkeys co-located in the same county during March through June 2015. Sera were retrieved in August 2016 from the University of Minnesota Veterinary Diagnostic Laboratory Infectious Agent Repository. Sera were tested for HPAI H5N2 antibodies by haemagglutination inhibition (HI) using virus A/Turkey/MN/ 9845-4/2015 H5N2 HPAI inactivated antigen (Tky/MN/15 H5N2) purchased from the United States Department of Agriculture (USDA) National Veterinary Services Laboratory and positive control pig antisera provided by USDA Agricultural Research Services from experimental infections of pigs with Tky/MN/15 H5N2. All sera were negative for H5N2 HI antibodies; however, one pig had an H5N2 HI titer of 1:10 and one pig had an H5N2 HI titer of 1:20. Of the 907 sera, 221 sera from pigs in 19 different herds were of suitable quantity and quality for additional IAV antibody testing using an IDEXX ELISA that detects antibodies to all IAV subtypes acquired through natural infection or vaccination. Seroprevalence as determined by ELISA was high (63%) and IAV antibodies were detected in 12 of the 19 herds. Whether the lack of H5 antibody detection is a result of no transmission from turkey to swine or possible heterosubtypic protection in swine from prior IAV exposure or vaccination remains in question. Regardless, we report the high current seroprevalence

of IAV in swine populations, the lack of seroepidemiological evidence of avian H5 influenza transmission to Minnesota pigs, and the need to further investigate novel IAV infections in pigs, determine how to prevent interspecies transmission, and devise methods to control the infection should that transmission event occur.

P7. Reassortant swine influenza A virus detected in Russia

Daria Danilenko, Komissarov A.B., Petrova P.A., Fadeev A.V., Komissarova K.S., Konovalova N.I., Eropkin M.Yu

[1]*Research Institute of Influenza, WHO-recognized National Influenza Centre, Ministry of Health of Russia, Russia*

Objectives: Swine influenza viruses (SIV) along with avian influenza viruses represent a constantly evolving threat for human and animal health. The pandemic potential of SIV was proven by the last pandemic. However, swine influenza surveillance in Russia is conducted poorly. In the last years the collaboration between the RII and veterinarians permitted to gain insight on the evolution of SIV in Russia. The objective of this study was to antigenically and genetically characterize reassortant SIV isolated in 2017 and analyze its potential threat to human health. **Methods**: SIV isolation in MDCK cell culture, typing, subtyping and antigenic analysis in haemagglutination inhibition assay (HAI) using rat polyclonal antisera against reference human and swine influenza viruses; antigenic cartography; next-generation sequencing of SIV, phylogenetic analysis. **Results**: The SIV strain was isolated in MDCK cells. Antisera raised against A (H1N1)pdm09 reacted poorly with the strain; however it was better recognized by the antisera raised against old seasonal A(H1N1) human influenza viruses (IV). Antigenic analysis of this isolate showed that it was distant from seasonal human IV that circulated in last 10 years before their extinction from human population in 2009. Next-generation sequencing of this strain revealed that it was a reassortant virus that contained all A (H1N1)pdm09 genes except the NA gene that was a typical swine N2 neuraminidase. However it had several amino acid changes in HA antigenic sites which probably can affect antigenic properties. This is the first time that such kind of reassortant A (H1N1)pdm09 viruses are isolated from swine in Russia though they have been detected in Europe in recent years. **Conclusion:** Previous studies conducted in the last 4 years of SIV in Russia have not identified such reassortant A (H1N1)pdm09 viruses. Considering the fact that they are antigenically different from A (H1N1)pdm09 viruses included into the vaccine such viruses may represent potential threat to human health especially for young children who are immunologically naive. Strengthening and widening of regular surveillance for SIV is needed to provide insight into the evolution of these viruses and contribute for better pandemic preparedness.

P8. Canine Influenza H3N2 Characterisation

Angela Danner[1], Karlie Woodard, Scott Krauss, Richard J. Webby, Stacey Schultz-Cherry, Victoria Meliopoulos

[1]*St. Jude Children's Research Hospital, USA*

Wild birds are considered the largest natural reservoir for Influenza A viruses (IAV) with only a few mammals (humans, horses, swine, and canines) having the ability to sustain outbreaks of IAV. In 2015 an outbreak of Canine Influenza Virus (CIV) H3N2 circulated in the United States. This caused public awareness and concern as many Americans

consider canines as companion animals, living alongside each other in their homes. Could CIV H3N2 be a risk to public health and have the potential for a zoonotic disease? In this study, pathogenicity, transmission, seroconversion and pathology in selected organs was evaluated using a ferret model with CIV's. Viruses chosen for evaluation were A/Canine/IL/41915/2015 H3N2 and A/Canine/Korea/CY009/2010 H3N2.

P9. Surveillance of swine Influenza in Spain: white pigs, free-range Iberian pigs and wild boars

Gustavo del Real, Paloma Encinas

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Spain

Spain is recognized by the OIE as a priority region for the surveillance of swine influenza because it meets the main risk factors for the spread and emergence of the influenza A virus in swine. Up to 10% of Spanish domestic pig population belongs to Iberian breed which are mostly bred in a free-range system. The wild-boar is other member of the Sus scrofa species which is susceptible to swine influenza viruses. Its population is very high in many regions of Spain and they frequently come in close contact with other domestic and wild animals. In this study, we aim to investigate the epidemiology of influenza infection and the characteristics of circulating influenza viruses in industrial farms of white pigs, in free-ranging Iberian pigs and in wild boars.
Regarding white pigs, during the period 2016/17 we have analysed 480 nasal swabs from 17 farms of 13 locations rich in intensive swine production. Thirty-nine samples (8.1 %) from 10 farms (58.8%) resulted RT-PCR +. Up to now, we have isolated 6 IVs from 6 different farms. By HI tests and subtype-specific RT-PCR, 5 of them were subtyped as Eurasian avian-like sw-H1N1. Whole genome data of these viruses is available for further genetic and phylogenetic analysis.
In relation to Iberian pigs, 577 sera from 22 farms have been analysed. By bELISA, 328 animals (56,8%) and 15 herds (68.2%) were positive. Subtype-specificity distribution of IV-positive sera was: 47.2% avian-like H1N1, 36.1% pdm09 H1N1, 27.8% human-like H3N2 and 5.5% human-like H1N2. A significant number (27.8%) of sera were bELISA (NP-specific)-positive but HI-negative for current SIVs. To elucidate the antigenic specificity of these sera, we are using different IV strains in HI assays as well as microarrays and ELISA with multiple recombinant HAs. Seroprevalence of SIV in wild boar populations was 10.8% (56/518) ranging from 0% to 53.3% depending on the region. Nasal swabs from free-range Iberian pigs and wild boars were negative for SIV by RT-PCR. We have observed a high antigenic cross-reactivity between Spanish viral isolates collected in 2004 and current isolates that indicate a slow antigenic variation over a period of 13 years.

P10. Surveillance of influenza A viruses in Western Canadian pigs and people

Susan Detmer, Murcia-Rodriguez DM, Harding JCS, Kirychuk S

University of Saskatchewan, Canada

Influenza A viruses (IAV) that can infect both humans and pigs pose a threat to public health as well as health and economic burdens for pig production. Various studies have shown the link between swine IAV (IAV-S) and human IAV indicating that swine workers

and their families have a greater probability of infection compared to the general population. Therefore, it is important to include swine workers in studies of IAV-S on endemically infected farms. Surveys of clinical symptoms, nasal swabs (NSSW) and oral swabs (ORSW) were collected from 26 swine workers (n=130) on 11 farms in Western Canada where 10 pig nasal swabs were being collected for an active surveillance pilot project. Samples collected monthly between October 2015 and May 2016 were tested by matrix RT-qPCR (Ct < 35 is positive). No human samples were positive; however, 5/130 NSSW (3.8%) and ORSW 6/130 (4.6%) had suspect level Cts (Ct 35-40) and 2 of these were H1 subtype. Swine NSSW were pooled, tested and subtyped; 42/165 (25.5%) pools were positive with 27 (75%) of these subtyped as H1N2. While trends were observed, no significant correlation was found between clinical symptoms and RT-qPCR results. In humans with suspect level Cts, there were significant correlations (*Spearman; P<0.05*) between suspect positive ORSW results and muscle aches and runny nose. Pearson correlation examining the pig NSSW pools demonstrated significant correlations between Ct values of individual pools and the pool averages. IAVs in humans are usually associated with mild clinical symptoms, similar to what we found in our study in humans with suspect level Ct values. While 9 humans had suspect level RT-qPCR results (5 suspect NSSW and 6 suspects ORSW), there was insufficient virus present in these samples to sequence and determine if there was transmission of IAV-S between swine to humans. However, one of the suspect samples was from a worker who was the only epidemiological link between two farms where an alpha H1N2 virus moved between pigs. Further research is needed with improved sample collection methods and increased sample size. This research may be of importance for early detection of IAV-S strains that infect humans. Larger projects involving more swine workers and farms spanning multiple geographic regions are important when looking at the prevalence of IAV-S, risk factors for both humans and pigs, and the development of preparedness plans of IAV-S infections in the broader human population.

P11.Novel approaches for influenza surveillance in swine breeding herds

Jorge Garrido Mantilla, Julio Alvarez, Marie Culhane, Montserrat Torremorell

College of Veterinary Medicine, University of Minnesota, USA

Surveillance of influenza A virus (IAV) is central to the control of influenza in pigs and the prevention of zoonotic infections. However, routinely and readily detecting influenza infections can be challenging specially in endemically infected herds. In this study, we compared novel sampling strategies to assess the best strategy for detecting and isolating IAV in swine breeding herds. The strategies evaluated were nasal swabs (NS), nasal wipes (NW), oropharyngeal swabs (OS), oral fluids (OF), surfaces wipes (SW), sow udder skin wipes (UW), airborne particle deposition wipes (APD) and air. Sampling was conducted in piglets prior to weaning or their environment in 6 breeding herds. All samples were tested by IAV matrix gene rRT-PCR and results considered positive if ct value was < 35. A subset of rRT-PCR positive samples was cultured for virus isolation using MDCK cells. The optimum sample type for IAV detection was identified using McNemar test ($p<0.05$). IAV was detected in 4 out of 6 breeding herds. Out of the 40 samples collected in piglets in IAV positive herds, 78% (31/40) of OS, 55% (22/40) of NS and 53% (21/40) of NW were rRT-PCR positive ($p=0.012$). 78% (31/40) of UW were positive compared with 60% (24/40) of SW and 86% (6/7) of OF ($p=0.035$). IAV was detected in 100% (40/40) of air samples compared with 88% (35/40) of APD. IAV was isolated from 78.% (40/51) of OS, 63% (34/54) of NS, 65% (37/57) of NW, 48% (10/21) of SW, 75% (18/24) of UW, 17% (1/6) of OF, 29% (7/24) of APD and 34%

(9/26) of air samples. Use of UW and OS and to a lesser extent OF and SW significantly increased the likelihood of detecting IAV by rRT-PCR compared to NS (baseline category) ($p<0.01$). In this study, OS was the optimum sample type for both detecting and isolating IAV from pigs. UW of lactating sows was also a sensitive method to identify positive litters and yielded viable IAV. Sampling the environment also appeared to be a good approach to detect IAV since IAV was readily detected from surfaces and air but yielded fewer isolates than OS and UW. This study provides new information on sampling approaches to detect influenza in breeding herds.

P12. Verdinexor (KPT-335) shows antiviral activity against multiple emerging influenza strains

Sharon Tamir[1], **Savanna Gornisiewicz**[1], Rebecca Dewar[2], Sharon Tamir[1], Paul Digard[3], Douglas Widman[1], Sharon Shacham[1]

[1] Karyopharm Therapeutics Inc, USA; [2] NHS Lothian, Scotland, UK; [3] University of Edinburgh, Scotland, UK

Objective: Influenza is a global health concern. Often these viruses emerge through recombination of strains from pig, equine, and bird in addition to humans. Many of these emerging strains, such as H1N1 have caused major pandemic outbreaks, spreading to millions of people worldwide. There is a high unmet need to find influenza therapies that are efficacious against various strains of influenza while avoiding drug resistance. Verdinexor (KPT-335), a well-tolerated orally bioavailable inhibitor of the nuclear export protein, exporting 1 (XPO1), reduces viral replication by inhibiting the nuclear export of influenza cargos like viral ribonucleoproteins (vRNP). Additionally, therapeutic targeting of XPO1, a host protein hijacked by influenza virus to facilitate replication, makes development of resistance unlikely, in contrast to current antiviral therapies that target viral proteins. **Methods:** Viral gene expression after treatment with Verdinexor was measured observed and quantified by immunofluorescence microscopy. Efficacy was determined by plaque reduction assay in MDCK cells. Verdinexor was tested against a panel of human and animal isolates of IAV by measuring endpoint titres of multi-cyclic replication assays in A549 cells. Verdinexor was tested against a swine (H1N1), duck (H4N6, H5N3) and an equine (H3N8) derived virus as well as a human isolate (H3N2). EC50 was determined by plaque reduction assay and cell viability (CC50) by luminescent measurement of ATP content. To determine potential resistancy, NP-mutant virus susceptibility was tested by multiple phenotypic assays including plaque reduction assays, dose-response inhibitions and analysis of NP intracellular localization. Resistant viruses were developed in cells and virus genome was sequenced. **Results:** Verdinexor reduced plaque number in MDCK cells by ~60% at a concentration of 0.3ï□M and completely inhibited plaque formation at 1ï□M with a subsequent IC50 value of 0.18ï□M. Verdinexor displayed dose-dependent inhibition of virus replication with IC50 values ranging between 4-30nM, with concomitant SI in the range of 87-650. Some Verdinexor resistance occurred by single amino acid changes in virus NP however; the drug resistant viruses were attenuated and displayed increased susceptibility to human immune factor MxA. **Conclusions:** These results suggest that Verdinexor is potentially an effective antiviral and may be a successful therapeutic agent against many strains of IAV. Although resistance could be generated over time, the single point mutation in NP causes a viral fitness defect and thus it is unlikely for Verdinexor resistance to emerge. Accordingly, Verdinexor provides a novel and effective approach to the treatment of potentially emergent influenza virus infections.

P13. Low Prevalence of Enzootic Equine Influenza Virus among Horses in Mongolia

Gregory G. Gray, Alexandra Sack, Ulziimaa Daramragchaa, Maitsetseg Chuluunbaatar, Battsetseg Gonchigoo, Boldbaatar Bazartseren, Nyamdorj Tsogbadrakh

Division of Infectious Diseases, & Global Health Institute, Duke University, USA; Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

Horses are critically important for Mongolian herders' livelihoods, providing transportation and food products, and playing important cultural roles. Equine influenza virus (EIV) epizootics have been frequent among Mongolia's horses, with five occurring since 1970. **Objectives:** We sought to estimate the prevalence for EIV infection among horses and Bactrian camels with influenza-like illness between national epizootics. **Methods/results:** In 2016-2017, active surveillance for EIV was periodically performed in four aimags (provinces). Nasal swabs were collected from 680 horses and 131 camels. Seven of the horse swabs were "positive" for qRT-PCR evidence of influenza A (Ct value < 38). Two more were "suspect positive" (Ct value > 38 and < 40). These nine specimens were collected from four aimags. None of the camel specimens had molecular evidence of infection. Despite serial blind passage in Madin-Darby Canine Kidney cells (MDCK) cells, none of the nine horse specimens yielded an influenza A virus. None of the 131 herder households surveyed had recently vaccinated their horses against EIV. **Conclusions:** It seems likely that sporadic EIV is enzootic in multiple Mongolian aimags. This finding, the infrequent use of EIV vaccination, periodic prevalence of highly pathogenic avian influenza, and the mixing of domestic and wild equid herds suggest that Mongolia may be a hot spot for novel EIV emergence.

P14. Prospective Surveillance for Influenza A Virus in Chinese Swine Farms

Gregory G. Gray, Benjamin D. Anderson, Mai-Juan Ma, Guo-Lin Wang, Zhen-Qiang Bi, Bing Lu, Xian-Jun Wang, Chuang-Xin Wang, Shan-Hui Chen, Yan-Hua Qian, Shao-Xia Song, Min Li, Teng Zhao, Meng-Na Wu, Laura K. Borkenhagen, Wu-Chun Cao.

Division of Infectious Diseases, & Global Health Institute, Duke University, Durham, USA; Duke-Kunshan University, Kunshan, China; Duke-NUS Medical School, Singapore

Pork production in China is rapidly increasing and swine production operations are expanding in size and number. However, the biosecurity measures necessary to prevent swine disease transmission, particularly influenza A viruses (IAV) that can be zoonotic, are often inadequate. Despite this risk, few studies have attempted to comprehensively study IAV ecology in swine production settings. **Objectives:** Here, we present environmental and animal sampling data collected in the first year of an on-going five-year prospective epidemiological study to assess IAV ecology as it relates to swine workers, their pigs, and the farm environment. **Methods/results:** From March 2015 to February 2016, we collected 396 each of environmental swab, water, bioaerosol, and fecal/slurry samples, as well as 3300 pig oral secretion samples from six farms in China. The specimens were tested with molecular assays for IAV. Of these, 46 (11.6%) environmental swab, 235 (7.1%) pig oral secretion, 23 (5.8%) water, 20 (5.1%) bioaerosol, and 19 (4.8%) fecal/slurry specimens were positive for influenza A by qRT-PCR. Risk factors for IAV detection among collected samplers were identified using bivariate logistic regression. **Conclusions:** Overall, these first-year data suggest that

IAV is quite ubiquitous in the swine production environment and demonstrate a strong agreement between the different types of environmental sampling used. Given the mounting evidence that some of these viruses freely move between pigs and swine workers, and that mixing of these viruses can yield progeny viruses with pandemic potential, it seems imperative that routine surveillance for novel IAVs be conducted in commercial swine farms.

P15. Dynamics of influenza virus of swine circulating in pig farms in Thailand

Junki Mine[1, 2], Sujira Parchariyanon[3], Nobuhiro Takemae[1, 2], Prakit Boonpornprasert[3], Namfon Ubonyaem[3], Bandit Nuansrichay[3], Taichiro Tanikawa[1, 2], Yuko Uchida[1, 2], Ryota Tsunekuni[1, 2], Takehiko Saito[1, 2]

[1]National Institute of Animal Health, National Agriculture and Food Research Organization, Thailand-Japan Zoonotic Diseases Collaboration Center, Japan;[2] Thailand-Japan Zoonotic Diseases Collaboration Center, Japan; [3]National Institute of Animal Health, Thailand

Surveillance activities on influenza A virus of swine (IAV-S) have been accelerated not only for moderating the economic loss to the swine industry but also for monitoring newly-generated IAVs-S with a potential of next pandemic. In Thailand, we previously demonstrated that IAVs-S of H1N1, H3N2, and H1N2 had circulated in farms studied. Their gene constellations are highly complex as they were reassortants among either classical swine viruses, Eurasian avian-like swine viruses, pandemic H1N1 viruses A(H1N1)pdm09 or human-like H3N2 viruses. In the present study, we have carried out longitudinal virological monitoring in four pig farms in Thailand from 2011 to 2017 to understand dynamics and evolutions of virus in those farms. **Materials and Methods**: Nasal swabs were collected periodically from four pig farms B, C, D and O located in the central region of Thailand from 2011 to 2017. Entire genome of IAVs-S isolated in this study were sequenced and phylogenetically analysed. Haemagglutination inhibition test (HI test) was used for antigenic analysis. In total, 169 viruses consisting of 82 H1N1 viruses and 87 H3N2 viruses from 3790 nasal swabs were isolated. A(H1N1)pdm09 and/or human-like H3N2 viruses, possessing internal genes of A(H1N1)pdm09 virus, were isolated in four pig farms. HA segments of H3N2 viruses in farm B and H1N1 viruses in farm D were phylogenetically divided into two distinguishable subclades respectively, suggesting that two independent introductions of the viruses into respective farm. H3N2 viruses isolated in farm C and O, which formed one cluster, showed similar antigenicity and were retained over one year at each farm. HA segments of H1N1 viruses in farm B and C, and H3N2 viruses in farm D formed one clade, respectively, with accumulating substitutions to change the reactivity to sera or monoclonal antibodies. HI test using monoclonal antibodies demonstrated alternation of reactivity to some of them with H1N1 viruses in farm B over 5 years. Accumulation of the amino acid substitutions might help viruses to escape immune pressure, and circulate in a pig farm for long period.

P16. The emergence and evolution of novel reassortant influenza A viruses in canines in southern China

Martha Nelson, Ying Chen, Nídia S. Trovão, Guojun Wang, Weifeng Zhao, Ping He, Huabo Zhoua, Yanning Mo, Zuzhang Wei, Kang Ouyang, Weijian Huang, Adolfo García-Sastre

Fogarty International Center, USA

The capacity of influenza A viruses (IAVs) to host-jump from animal reservoir species to humans presents an on-going pandemic threat. Birds and swine are considered major reservoirs of viral genetic diversity, whereas equines and canines have historically been restricted to one or two stable IAV lineages with no transmission to humans. Here, by sequencing the complete genomes of 16 IAVs obtained from canines (CIVs) in southern China (Guangxi autonomous region) during 2013-2015, we demonstrate that the evolution of CIV in Asian dogs is becoming increasingly complex, potentially presenting a threat to humans. First, two reassortant H1N1 virus genotypes were introduced independently from swine into canines in Guangxi, including one genotype previously associated with a zoonotic infection. The genomes contain segments from three lineages that circulate in swine in China: North American triple reassortant H3N2, European avian-like H1N1, and pandemic H1N1. Importantly, the swine-origin H1N1 viruses have transmitted onward in canines and reassorted with the CIV-H3N2 viruses that circulate endemically in Asian dogs, producing three novel reassortant CIV genotypes (H1N1r, H1N2r, and H3N2r). All 16 CIVs from this study were collected from pet dogs with respiratory symptoms at veterinary clinics, but dogs also are raised for meat in Guangxi, and stray dogs roam freely, creating a more complex ecosystem for CIV evolution. Further surveillance is greatly needed to understand the full genetic diversity of CIV, how the viruses are emerging and persisting in the region's canine populations, and the zoonotic risk as the viruses continue to evolve.

P17. Genetic diversity of influenza A viruses circulating in pigs between winter and summer in a Minnesota live animal market, 2012-2013

Jayaveeramuthu Nirmala[1], Jeff Bender[1], Marie Culhane[1], Martha Nelson[2], Srinand Sreevatsan[3], Ruth Lynfield[4] and Montserrat Torremorell[1]

[1]*Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, USA;* [2] *Fogarty International Center, National Institutes of Health, USA;* [3]*Veterinary Medical Center, Michigan State University, USA;* [4] *Minnesota State Health Department, USA*

We conducted surveillance among swine and of the environment for influenza A viruses (IAVs) at a live animal market in Minnesota that has been epidemiologically associated with variant influenza cases in humans. Swine are from multiple commercial sources, and upon purchase are slaughtered on site. IAV surveillance which included pig oral fluid and tissue samples, air and hand contact surfaces, was conducted during both the winter (cold) and summer (warm) seasons in order to evaluate the persistence of IAV between seasons. Forty IAVs from summer and 122 isolates from winter were isolated, sequenced and genetically compared. Overall, IAV was found to be prevalent in swine and the environment in both summer and winter seasons with similar isolation rates obtained from swine samples (65% vs. 63%), and lower, although detectable, rates obtained from air samples collected in the summer (26% vs. 67%). Two subtypes, H3N2 and

H1N2 co-circulated in summer, while 3 subtypes H3N2, H1N2 and H1N1 were detected in winter. H3N2 was the most prevalent subtype in both seasons, followed by H1N2. Genetically diverse viruses with multiple gene constellations were isolated from both the winter and summer with a total of 19 distinct genotypes identified. Comparative phylogenetic analysis of all 8 genes of 40 virus isolates from summer and 122 from winter revealed that the summer and winter isolates were the result of separate and genetically distinct introductions and that the viruses originated primarily from commercial swine sources. There was no concrete evidence for persistence or continuous transmission of the viruses from winter to summer in this live animal market. Since the facilities may occasionally be emptied and are, by definition, terminal markets, termination of transmission through removal of the swine host could be reasons why the winter viruses die out. Interestingly, these markets constantly receive swine from multiple origins, which might facilitate the continual introduction of genetically distinct IAVs. Live animal markets may potentiate exposure to a wide variety of variant IAV throughout the year.

P18. Can recommendations for reducing zoonotic transmission of influenza A viruses from swine impact human attitudes and behaviours?

Jacqueline M. Nolting, Andrew S. Bowman

The Ohio State University, USA

Since 2011, there have been 425 cases of variant influenza A virus (IAV) reported in the United States, many of which were associated with youth swine exhibition. In an effort to mitigate risk associated with exposure to IAV in swine through these events, the recommendation document "*Measures to Minimize Influenza Transmission at Swine Exhibitions*" was developed to be used by show organizers, volunteers, and exhibitors. These recommendations are updated and released each year; however, it is not clear if youth swine exhibitors are aware of the recommendations; support the recommendations; and would be willing to practice recommended behaviours. Therefore, a cross-sectional survey method was used to understand swine exhibitor perceptions and their adoption of swine production practices aimed at reducing the transmission of IAV at the human-animal interface. The instrument created consisted of 11 recommendations put forth in the "*Measures*" document. Each statement was followed by three to six statements regarding the participant's perception of the recommendation, their opinion of their ability to implement the recommendation, and their current behaviour related to the recommendation. In addition, the survey asked participants their state of residence and the number of shows they would attend in 2017. In all, 155 participants who showed swine on a regular basis ($\bar{x}$=11 shows per year), from at least 18 states within the US, completed the survey. At least 67% of participants believed each statement was a good recommendation, with 6 of 11 recommendations being supported by >90% of participants. When asked if recommendations could be implemented, 65-94% of respondents agreed, and 21-89% of participants had already implemented each recommendation, respectively. Recommendations that have been widely adopted by respondents include; establishing a relationship with a veterinarian (89%) and vaccinating swine for IAV (83%). The recommendations not being implemented commonly by respondents include; not eating and drinking in animal areas (79%), reporting sick pigs to show organizers (57%), and isolating animals when they are taken home after a show (50%). Although significant efforts have been made to increase signage at swine exhibitions warning of risks associated with eating/drinking in animal areas, a majority of respondents report eating/drinking in the barn and are unwilling to

change their behaviours. This study provides evidence that developing and disseminating static recommendations to reduce zoonotic disease transmission is not enough to change human behaviour and interactive methods must be deployed to preventive future variant IAV infections associated with swine exhibitions.

P19. Different efficacy of inactivated pandemic 2009 H1N1 influenza A virus vaccines after homologous infection in ferrets.

Alejandro Nuñez[1], B. Vidaña, F. Garcon[2], D Major[3], I.H. Brown[2], M. Zambon[4] and S.M. Brookes[2]

[1]Animal and Plant Health Agency APHA-Weybridge, Pathology Department, UK, [2] Animal and Plant Health Agency, APHA-Weybridge, Virology Department, UK, [3] National Institute for Biological Standards and Control, UK,[4] Public Health England, UK

Introduction and objectives: Since its emergence, the (H1N1)pdm09 influenza A virus (IAV) has been established worldwide and replaced the previous seasonal H1N1 viruses, becoming one of the most isolated strain in recent years. The extent to which (H1N1)pdm09 influenza vaccines prevent viral infection and disease remains poorly understood. Hence, we evaluated the efficacy of two (H1N1)pdm09 influenza human vaccines in ferrets. **Materials and Methods:** Twelve influenza naive ferrets were divided in two groups (V1 and V2) and immunised intramuscularly with two different A/California/07/2009 derived inactivated vaccines. Six weeks later, all animals were intranasally challenged with 106.5 TCID50 of the A/England/195/09 (H1N1)pdm09 isolate. A non-vaccinated (NV) challenged group of 6 animals was used as an infection control. Clinical signs, lung histopathology, viral quantification and antibody responses were evaluated. **Results:** The V1 group showed reduced viral loads, milder clinical signs and histopathological scores. In contrast, V2 vaccinated animals exhibited higher clinical scores and higher viral shedding than the V1 groups and similar to the NV control group. V2 vaccinated animals presented slightly more severe histopathological lesional scores at 5 days post infection in correlation with slightly higher presence of IAV IHC positive cells in the lungs. **Conclusions:** There were important qualitative differences in the performance of both inactivated vaccines in relation to protection against challenge with a novel virus in a naive animal (ferret) model of human disease. V1 vaccine limited and controlled viral shedding and reduced lower respiratory tract infection, in contrast, V2 vaccine did not protect against infection and appeared to maintain viral shedding and delay lower respiratory viral infection, resulting in similar histopathological lesions that the NV control group.

P20. A new polymorphism in the PB1-F2 protein of Argentinean strains of equine influenza virus

Cecilia Olguín Perglione[1], Maria Barrandeguy[2]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA). Instituto de Virología, Argentina, [2]Escuela de Veterinaria, Universidad del Salvador, Argentina

Influenza A virus (IAV) genome segment 2 (PB1) encodes the PB1 subunit of the trimeric RNA polymerase. The PB1-F2 is a polypeptide of numerous IAV isolates encoded by an alternative ORF of this segment. This protein could induce apoptosis, promote inflammation and up regulate viral polymerase activity, contributing to enhance the pathogenicity of the virus. The PB1-F2 possesses a predominantly mitochondrial

localization via a mitochondrial targeting sequence (MTS), which is located from amino acid (aa) 63 to 75. Previous reports described that PB1-F2 of H3N8 equine influenza virus (EIV) strains contained 90 and 81 aa in length for viruses identified before and after 1997 respectively. The aim of this work is to analyse the molecular characteristics of genome segment 2 of the EIV detected in Argentina. The nucleotide and deduced aa sequences for the complete PB1 gene of 25 EIV, detected during multiple outbreaks of equine influenza occurred in Argentina between 1993 and 2012, were analysed. The phylogenetic tree was inferred by the maximum likelihood method, along with 106 reference PB1 gene sequences available at the Influenza Research database. The Argentinian EIV groups into three monophyletic clades, sustained by high support values, the South American (SA) clade 1 (n=5), the SA clade 2 (n=8) and the Florida clade 1 (n=12), which are made up of strains detected from 1993 to 1996, 1997 to 2006, and in 2012, respectively. The deduced aa sequences of the PB1-F2 of strains belonging to the SA clade 2, showed a polymorphism due to a stop codon at position 238, yielding a polypeptide of 79 aa in length. The Argentinian strains in the SA clade 1 and Florida clade 1 have 90 and 81 aa respectively, in accordance with previous data. The fact that EIV detected in Argentina between 1993 and 2012 grouped into three different monophyletic clades, suggests three distinct introductions of the virus into the country and an independent, regional evolution. The polypeptide of 79 aa was not previously described in EIV. Although it is shorter, it retains the MTS, so it could keep its functionality and mitochondrial localization. Further studies are needed to determine if this change has some biological consequence.

P.21 Validation of real-time RT-PCR protocols for sub-typing European swine influenza viruses and differential detection of reassortant H1N2 swine influenza virus in pigs in Great Britain

Scott M. Reid, Christine Russell, Jayne L. Cooper, Vivien Coward, Helen E. Everett, Elizabeth Coney, Alexander M. P. Byrne, Susanna Williamson, Sharon M. Brookes, Ian H. Brown

Animal and Plant Health Agency-Weybridge, UK

Objectives: Swine influenza virus causes respiratory disease and productivity loss in pigs world-wide. Three sub-types (H1avN1, H1N1pdm09, H1huN2) of swine influenza A viruses (swIAVs) including reassortants are currently found in pigs in Great Britain (GB). Since 2010, an H1N2-H1N1pdm09 virus reassortant has been detected; H1 haemagglutinin (HA) and N2 neuraminidase (NA) surface glycoproteins with the H1N1pdm09 internal cassette. Surveillance of swIAV sub-types is important for epidemiological investigations, vaccination, pig health and welfare, and public health. Real-time reverse transcription polymerase chain reaction (RRT-PCR) assays were developed to improve the sensitivity and speed of swIAV sub-typing. These protocols cannot, however, specifically identify reassortant H1N2 (H1N2r) swIAVs circulating in GB, and both the conventional H1huN2 and H1N2r now co-circulate. A RRT-PCR for differential detection of H1N2r in the GB pig population was therefore required for use in conjunction with sub-typing RRT-PCR assays. **Methods:** Simplex RRT-PCR assays for detecting H1av and H1hu, or H3, and duplex assays to detect N1 (N1av) or N2 were assessed on influenza A PCR-positive, mostly virus isolation (VI)-negative, field material from 2012-2017 from GB pigs. An RRT-PCR to specifically detect the nucleoprotein (NP) internal gene of H1N1pdm09, incorporating a re-designed locked nucleic acid probe, was developed to differentiate conventional H1N2 from H1N2r swIAVs. **Results:** Sub-typing identified H1N2 and H1avN1 swIAVs in 35.6% (n=104) swabs and

tissues, while in 36.5% only HA or NA were detected and in 27.9% no sub-type was detected. The H1N1pdm09-NP RRT-PCR assay correctly identified the H1N1pdm09 NP gene segment in H1N2r viruses from four H1N1pdm09 control viruses and from 12 conventionally-typed H1N2 viruses. **Conclusions:** Rapid sub-typing of previously uncharacterized swIAVs directly from field material without VI or nucleotide sequencing was successful, although assay sensitivity requires improvement to achieve subtyping for a greater proportion of field material. By building on the subtyping RRT-PCR protocols, the H1N1pdm09-NP RRT-PCR assay will provide added value for influenza A surveillance in GB pigs.

P22. Assessment of Influenza D virus in swine: first serological evidence for exposure of breeding sows in France

Gaëlle Simon, Stéphane Gorin, Christelle Fablet, Stéphane Queguiner, Frederic Paboeuf, Mariette Ducatez, Séverine Herve, Nicolas Rose

French Agency for Food, Environmental and Occupational Health & Safety- ANSES, Ploufragan-Plouzané Laboratory, Swine Virology Immunology Unit, France

Bovine represent the reservoir to influenza D virus (IDV) but this virus was also isolated from pigs in the USA and Italy. In order to provide additional information about its host range, we started to investigate IDV circulation among pigs in France. First, SPF pigs were inoculated with a bovine IDV to produce reference reagents for diagnosis in swine. A specific haemagglutination inhibition (HI) assay was developed using a swine IDV isolate as an antigen and a swine antiserum as a positive control. Sera from naive SPF pigs were used as negative controls. A duplex real-time RT-PCR amplifying IDV PB1 and β-actin genes was developed for IDV detection in clinical samples. Serological tests were first conducted on 1048 archived sera obtained from breeding sows sampled between January 2014 and June 2015 in 35 farrow-to-finish herds (30 sows/herd) with respiratory disorders and located in Brittany, the highest pig populated area in France. Thirty-one sera (2.9%) originating from six herds (17.2%) contained IDV-specific antibodies. In four of them, only 1 or 2 sows tested positive with HI titers of 20. In the two others (A and B), 22/30 (73.3%) and 4/30 (13.3%) sera tested positive, respectively, with HI titers 20-160. New sampling in March 2017 showed that 2/15 (13.3%) sows from herd A and 1/30 (3.3%) sow from herd B tested positive again, with HI titers 20-80. The 30 fattening pigs sampled in each herd were seronegative. In September 2017, a respiratory outbreak occurred in herd A on 15 week-old pigs but IDV was not detected in nasal swabs taken on 10 animals with hyperthermia. Two other banks of archived sera obtained from growing pigs in Breton herds tested negative: i) 300 sera taken November 2013-February 2014 in 10 herds on 16 or 22 week-old pigs (15 pigs/batch); ii) 279 sera obtained in 31 farrow-to-finish herds between 2012 and 2016 from post-weaning to slaughtering. In 2017, 119 sera from 10 or 24 week-old pigs from two herds located in South-western France also tested negative. In addition, virological investigations conducted in 2016-2017 on growing pigs with acute respiratory syndrome gave negative results in IDV RT-PCR (255 nasal swabs from 69 herds). Whereas IDV circulation was not highlighted among French growing pigs, these investigations provide the first serological evidence that breeding sows have been exposed to IDV in Brittany, raising questions about its prevalence in this population.

P23. Antigenic distance of swine H3N2 and human influenza A virus seasonal strains as an indication of risk to human populations

Carine K. Souza [1], Tavis K. Anderson[1], Divya Venkatesh[2], Marcus Bolton[1], Susan E. Detmer[3], Ignacio Mena[4], Eugenio J. Abente[1], Nicola Lewis[2], Adolfo García-Sastre[4], Amy L. Vincent[1]

[1] National Animal Disease Center, USDA-ARS, USA; [2] University of Cambridge, UK; [3] University of Saskatchewan, Canada; [4] Icahn School of Medicine, NY, USA.

H3N2 influenza A viruses (IAV) cause seasonal epidemics in humans. Globally, human-to-swine interspecies transmission events have repeatedly occurred, leading to H3N2 lineages that also circulate in pigs and to increased viral diversity in pig populations. In North America, a human H3N2 IAV introduction into swine occurred in the late 1990s and genetically evolved into a stable swine lineage named cluster IV (C-IV). A 2010 human seasonal H3N2 IAV introduction was more recently detected in U.S. swine, and has now become the predominant H3N2 IAV lineage in the U.S. pig population. This lineage is referred to as human-like to differentiate from C-IV. Both C-IV and human-like swine H3N2 have been associated with variant H3N2 (H3N2v) detections and illness in humans. These events highlight the potential for re-emergence of IAV from swine back into humans. Here, we quantified antigenic distance between human seasonal and endemic s wine H3N2 IAV with haemagglutination inhibition (HI) assays and antigenic cartography. We used a panel of monovalent anti-sera raised in pigs against human seasonal H3N2 vaccine strains from 1973â€“2013 and contemporary swine strains from U.S., Mexico, Canada and Denmark. Antigenic distances between viruses were calculated in antigenic units (AU), in which 1 AU is equivalent to a 2-fold loss in HI cross-reactivity. Contemporary C-IV swine strains were antigenically closer to human strains from the late 1980s and early 1990s, from the period when the initial human-to-swine spill over occurred, with distances between human and swine strains of 2.4-5.2 AU for US, 1.0-5.9 AU for Mexico, and 2.6-6.4 AU for Canada swine IAV strains. However, the C-IV lineage swine viruses were at least 5 AU from the latest decade of human H3N2 IAV vaccine strains. In contrast, 2010 U.S. and 2004 Danish human-like swine IAV demonstrated substantial antigenic distance from human seasonal H3N2 IA V vaccine strains isolated prior to 2007 (>5.0 AU), and were more closely related to human seasonal strains from the last decade (0.4-4.3 AU). Swine anti-sera panel created using human-origin IAV may be a surrogate for human population immunity and could potentially identify swine influenza A H3N2 viruses for further risk characterization by performing HI tests against age-stratified human sera, receptor binding assays, or in other risk models.

P24. Antigenic differences among H1 influenza A viruses circulating in pig population in Vietnam between 2010 and 2017

Nobuhiro Takemae[1,2], Phuong Thanh Nguyen[3], Tien Ngoc Nguyen[4], Thanh Long To[5], Vu Tri Le[3], Tho Dang Nguyen[5], Tung Nguyen[4], Vu Phong Pham[3], Hoa Thi Do[5], Hung Van Vo[3], Quang Vinh Tin Le[3], Vui Thi Dam[5], Diep Thi Nguyen[4], Yuko Uchida[1,2], Takehiko Saito[1,2,6]

[1]Division of trans-boundary animal diseases, National Institute of Animal Health, NARO, Japan, [2] Thailand-Japan Zoonotic Diseases Collaboration Center, Thailand;[3] Center for Veterinary Diagnostics, Regional Animal Health Office No. 6, Department of Animal Health, Vietnam;[4] Epidemiology Division, Department of Animal Health, Vietnam;[5] National Centre for Veterinary Diagnostics, Department of Animal Health, Vietnam; [6] United Graduate School of Veterinary Sciences, Gifu University, Japan

Background: Understanding of the antigenicity of the influenza A viruses of swine (IAVs-S) is valuable for selection of appropriate vaccine strains. Our previous study demonstrated that three distinct haemagglutinin (HA) H1 lineages, North American triple-reassortant (TR), pre-2009 human-like (Hu) H1, and A(H1N1)pdm09 viruses had co-circulated from 2010 to 2015 in Vietnam. However, their antigenicities have not been well characterized. In this study, we examined the antigenicity of the Vietnamese H1 IAVs-S isolated from 2010 to 2017. **Materials and Methods:** A total of 236 H1 HA genes of the Vietnamese IAVs-S isolated from 2010 to 2017 was sequenced and phylogenetically analysed. The antigenic reactivities of H1 viruses were examined by HI assays. Hyperimmune antisera against representative Vietnamese IAVs-S were prepared in chickens. For comparison, antisera against seasonal human strains obtained from NIID, Japan, were also used. The antigenic differences were analysed using antigenic cartography. **Results:** Active surveillance from 2010 to 2017 yielded 236 IAVs-S of H1 subtype consisting of 97 A(H1N1)pdm09 viruses and 139 H1N2 viruses. In addition to pre-existing three H1 lineages by 2015, 2 European avian-like (EA) H1N2 IAVs-S were isolated in 2016. The averaged antigenic distances between TR and A(H1N1)pdm09 or Hu-H1 viruses were 3.6 and 4.0 antigenic units, respectively, but the distance between TR and EA viruses was 2.4 antigenic units. That is, TR, A(H1N1)pdm09, and Hu-H1 viruses belonged to antigenically distinct clusters identified by a k-means clustering algorithm, whereas the EA H1 virus was not antigenically distinguishable from the TR viruses. **Conclusions:** Our study demonstrated that 3 distinct antigenic groups of the IAVs-S exist in Vietnamese pig population, suggesting that such antigenic variation would make the design of vaccine difficult.

P25. Farm factors associated with influenza infection in piglets at weaning

Montse Torremorell, Fabian Chamba, Adam Schelkopf, Matthew Allerson, Bob Morrison, Marie Culhane

College of Veterinary Medicine, University of Minnesota, USA

Breeding herds play an important role in influenza infections because suckling piglets can maintain, diversify and transmit influenza A virus (IAV) to other farms. Understanding which farm factors drive influenza infection in piglets is critical to reduce the burden of influenza in swine. We evaluated the association of influenza infection in piglets at weaning with farm factors including farm characteristics, herd management practices, along with gilt- and piglet-specific procedures performed at the farm. A total of 83 breeding herds were voluntarily enrolled and agreed to share influenza diagnostic testing and farm data from July 2011 to March 2017 including data obtained via the administration of a survey. There were 12,814 samples from 2,989 submissions with 23% of samples and 30% of submissions testing IAV positive. Among all the factors evaluated, and considering the season-adjusted multivariable analysis, only sow influenza vaccination and gilt influenza status at entry were significantly (p-value<0.05) associated with influenza infection in piglets at weaning. Results from this study indicate that both influenza sow vaccination and introducing influenza negative replacement animals are important to decrease the risk of influenza infection in piglets at weaning.

P26.Influenza B Viruses in Taiwanese Pigs

Ching-Ping Tsai [1,2], Hsiang-Jung Tsai [2]

[1] *Agricultural Technology Research Institute, Taiwan;* [2] *National Taiwan University, Taipei City, Taiwan*

We isolated and identified 3 strains of influenza B virus (IBV) from pigs under active survey of influenza on Taiwanese swine populations in 2014. Genetic characterization of 3 swine strains of IBVs (sIBVs) revealed the highest identities (>99%) of nucleotide sequence for haemagglutinin (HA) and neuraminidase (NA) genes with one human IBV (B/Taiwan/113/2014), which collected on April 10, 2014, as well as for other 6 internal protein genes with the contemporary Victoria-like strains. Three sIBVs are grouped with B/Taiwan/13/2013 and B/Taiwan/113/2014 in the same cluster of the B/Brisbane/60/2008 genetic clade of Victoria lineage from the HA tree of 133 IBVs and the NA tree of 162 IBVs, respectively. In the phylogenetic trees of other 6 internal protein genes, 3 sIBVs are distributed into the sub-tree of B/Brisbane/60/2008-like viruses, respectively. When compared with B/Brisbane/60/2008, sequence variations in HA, NA, nucleo protein, matrix protein 1, non-structural protein 1, polymerase acidic protein, polymerase basic protein 1 and polymerase basic protein 2 genes have been identified in all sIBVs or some of them. In HA, all sIBVs carry amino acid substitutions in 3 of antigenic sites (V124A in the 120-loop, I146V in the 150-loop and N197D in the 190-helix). None of drug-resistant amino acids in NA was found on all sIBVs. Sero-epidemiological data from random selected pigs on swine farms under annual active surveys during 2007-16 indicated IBV-specific antibodies were detected in 31 (0.2%; 95%CI: 0.1%-0.3%) of 14,511 serum samples from 29 (3.1%; 95%CI: 2.1%-4.3%) of 943 farm visits. Seropositive cases for sIBVs scattered loosely and cumulatively over 11 (44.0%; 95%CI: 26.5%-63.0%) of 25 prefectures in 10 years. IBV infections in pigs had occurred in no more than 5 prefectures or 25.0% (95%CI: 10.3%-49.8%) of test prefectures every year except 2015. The findings mean that transmission of IBVs i s sporadic and accidental at the human-swine interface. Public health threats, however, might be considered because swine populations have served as a reservoir of IAV genes that have circulated in humans. Human IBVs would be kept in swine populations and provided as genetic sources for reassortment of novel influenza viral variants. Even if the risk is rare currently, therefore, potential avenues for newly emerging zoonotic influenza in the future might have been paved.

P27. Multiple reverse zoonotic transmission of A(H1N1)pdm09 viruses to pig population in Japan

Yuko Uchida[1], Nobuhiro Takemae[1,2], Toshihiro Ito[3], Hiroshi Ito[3], Yuko Uchida[1,2], Takehiko Saito[1,2,4]

[1] *Division of trans-boundary animal diseases, National Institute of Animal Health, NARO, Japan;* [2]*Thailand-Japan Zoonotic Diseases Collaboration Center, Thailand;* [3] *Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, Japan;* [4] *United Graduate School of Veterinary Sciences, Gifu University, Japan*

Background: Since the emergence of A(H1N1)pdm09 virus in human population in 2009, frequent reverse zoonotic transmission, human-to-pig transmission, of it has generated various genotypes of influenza A viruses of swine (IAVs-S) in many countries. To investigate existence of the reverse transmission of influenza A viruses in Japanese pig population, we phylogenetically characterised the IAVs-S isolated from our active and

passive surveillance in Japan. **Materials and Methods**: A total of 4,921 nasal swab samples of pigs were collected from 35 pig farms in 9 prefectures in Japan from 2015 to 2017. They were subjected to virus isolation, along with 21 specimens including tracheal swabs and respiratory organs from type A influenza diagnosed pigs from 2012 to 2017. Along with 24 IAVs-S submitted to NIAH-Japan for diagnosis, complete genome sequences of all the isolates were phylogenetically analysed to determine genetic origin of each gene segment. **Results:** Two hundred ninety-two IAVs-S including 65 H1N1, 217 H1N2, 8 H3N2, and 2 mixed subtypes (H1N1 and H1N2) were obtained. Entire genome of 34 H1N1 viruses were of A(H1N1)pdm09 virus origin, whereas the rest 31 H1N1 viruses and all of the H1N2 and H3N2 viruses were reassortants among the classical H1N2, Human-like H3 viruses and A(H1N1)pdm09 viruses. Those reassortants (257 strains) were divided into 5 genotypes, according to their genetic constellation. All of the reassortants retained PB2, PB1, PA, M and NS genes derived from A(H1N1)pdm09 viruses. Phylogeny of PB2 genes revealed that Japanese strains formed at least 20 distinct sub-clusters within A(H1N1)pdm09 cluster, suggesting frequent reverse zoonotic transmission of A(H1N1)pdm09 viruses into pig populations. The estimated number of A(H1N1)pdm09 introductions into pigs in each flu season were positively correlated with that of the incidence of human A(H1N1)pdm09 cases in Japan ($R2 = 0.93$, $P<0.01$). **Conclusions:** This study demonstrated the multiple reverse zoonotic transmissions of A(H1N1)pdm09 viruses as well as co-circulation of 6 genotypes of IAVs-S in Japan. It highlights the importance of hygienic control in pig farms during seasonal flu seasons in human population to prevent reverse zoonotic transmission into pigs.

P28. Cross-reactive antibodies to the major H1 swine influenza virus clades in human sera

Elien Vandoorn, Isabel Leroux-Roels, Geert Leroux-Roels, Kristien Van Reeth

Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium

The epidemiology of swine influenza viruses (SIVs) is complex due to multiple introductions of human influenza viruses in swine and the geographic separation of swine populations. As a result of this, multiple antigenically distinct H1 and H3 SIV clades are co-circulating and they differ between continents and regions. We and others have previously shown that humans lack antibodies against H3N2 SIVs for which the human precursor virus circulated before they were born. These SIVs may be reintroduced into the human population when population immunity drops below a certain critical threshold. Here we aim to conduct a similar study for H1 SIVs, which are more diverse than the H3 subtype. Previous investigations examining the reactivity of limited numbers of human serum samples against only a few H1 SIV clades generated confusing and sometimes contradictory results. Therefore, we have examined 500 sera from people aged between 0 and 100 years for reactivity against seven of the most important H1 SIV clades. Between August and December 2017, sera were collected from immune-competent patients, 50 subjects per age decade, at the Ghent University Hospital (Belgium). These sera were tested for the presence of haemagglutination inhibiting (HI) and virus neutralising (VN) antibodies to seven H1 SIVs, including classical swine (n=2), human-like (n=3) and Eurasian avian (n=2) lineages. The respective human H1 precursor viruses were also included. Results are pending and will be available in April 2018. The results of this survey may be useful for the assessment of zoonotic and pandemic risks of different H1 SIV clades for the human population and possibly for the selection of pre-pandemic vaccine strains.

P29. Reassortment and genetic diversity of swine influenza A viruses

Divya Venkatesh, Gaëlle Simon, Sévérine Herve, Emanuela Foni, Kristien Van Reeth, Maria Serena Beato, Alice Fusaro, Lars Larsen, Krzysztof Smietanka, Sharon Brookes, Ian H. Brown, Steve Essen, James Seekings, Susan Collins, Gustavo Real Soldevilla, Amy L. Vincent, Nicola S. Lewis

University of Cambridge, UK

Swine influenza presents a substantial disease burden for pig populations worldwide and poses a potential pandemic threat to humans. H1N1, H1N2 and H3N2 are the main subtypes of swine influenza A viruses (IAV). Phylogenetic analyses of the HA gene have identified three broad genetic lineages in currently circulating swine H1 strains: 1A: classical swine (including the 2009 pandemic H1N1), 1B: Human seasonal-like and 1C: Eurasian avian-like. The lineages reflect multiple introductions from both human seasonal strains (1A and 1B) and wild birds (1C) and have different (but partially overlapping) geographical distributions. Four introductions of H3 viruses into pigs have been identified, along with two additional lineages introduced into humans from swine. All pandemic and several epizootic events thus far have been associated with reassortment of influenza gene segments between different lineages. In this study, we determine the level of reassortment within and across the swine influenza lineages with isolates from currently circulating strains in different geographical areas across the world. We test for occurrence of selective sweeps and determine if lineage, geographic region or gene identity are associated with increased segment exchange between viruses. We find that the rates of reassortment in swine are lower than that seen in avian IAV lineages and more comparable to human IAV. We also measure the level of genetic diversity and rates of substitution in HA, NA and the internal gene segments in each lineage. And then attempt to correlate these metrics and map specific reassortment events with known instances of cross-species spill over to identify factors associated with such events.

P30.Sequence Auto-curation in the Influenza Research Database (IRD)

Yun Zhang, Catherine Macken, Sam Zaremba, Edward B. Klem, Richard H. Scheuermann

J. Craig Venter Institute, USA

The Influenza Research Database (IRD, www.fludb.org) is a US NIH/NIAID-funded, freely-available online bioinformatics resource for influenza virus data search, analysis and visualisation. One focus area of the IRD resource is to provide comprehensive, high quality sequence annotations for the research community. Toward this end, a suite of sequence annotation tools specifically tailored for influenza virus has been developed by the IRD team. Using these tools, all relevant influenza virus sequences in IRD have been annotated with HA H1/H5 clade classification, variant proteins (PB1-F2, PB1-N40, PA-N155, PA-N182, PA-X, M42 and NS3), and the presence or absence of Phenotypic Variant Types, in which particular sequence substitutions are predicted to give rise to phenotypic effect. In addition to these tools, a sequence auto-curation pipeline has been recently implemented in IRD. This pipeline detects potential sequencing artifacts by comparing query sequences with a curated reference alignment that captures natural variations in the appropriate type/segment/subtype category. Potential sequencing artefacts are flagged with regard to their location (conserved terminal sequence -CTS,

non-coding region -NCR or coding sequence -CDS) and type (extension, insertion, deletion or mutation). This pipeline is being used to automatically and comprehensively curate the sequences in IRD. As a result, users can not only view sequencing artifact flags in IRD, but also choose to include or exclude the problematic sequences in their analyses. Furthermore, for sequences with flags of NCR-extension, NCR-deletion, NCR-insertion, CTS-deletion, CTS-insertion or CTS-mutation, users are provided with the additional choice of using IRD-edited CDS sequences in their analyses. Besides being used for curation of IRD sequences, this auto-curation pipeline also allows users to detect potential sequencing artefacts in their own sequences. The robustness of the sequence auto-curation pipeline is monitored regularly, and the reference alignment is updated when new sequence patterns are validated in multiple sequences and sequencing laboratories. In summary, IRD provides high quality sequence data and associated metadata along with other analysis and visualization tools as part of its mission to facilitate research and development of diagnostics, prophylactics and therapeutics for influenza viruses.

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