2nd International Symposium on
Neglected Influenza Viruses
7th - 8th March 2013

Final Programme and Book of Abstracts
The Royal College of Physicians of Ireland
Number Six Kildare Street
Dublin 2, Ireland
WELCOME MESSAGE

We are delighted to welcome you to Dublin for the 2nd International Symposium on Neglected Influenza Viruses.

This Symposium will retain the format, and build on the success of the inaugural symposium held in Florida in 2010. This conference 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 nonhuman/nonavian influenza viruses.

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 transdisciplinary, 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 their 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 that most precious commodity – your time! We wish to acknowledge our professional conference organisers Event Plus for their pivotal role in the organisation of the meeting. We hope it is the first of many.

Thank you for joining us in Dublin. We hope that you have a memorable visit, that you make new contacts, are exposed to novel data and return home energised and enthusiastic to face the challenges afforded by these fascinating viruses.

Sincerely,

[Signatures of Co-Chairs]

CONFERENCE CO-CHAIRS

**Ann Cullinane MVB, PhD, MRCVS**  
Head of Virology Unit,  
Irish Equine Centre  
Johnstown, Co. Kildare, Ireland.

**Debra Elton PhD**  
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Animal Health Trust  
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College of Public Health and Health Professions  
Professor, Department of Infectious Diseases and Pathology,  
College of Veterinary Medicine  
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Ghent University
Merelbeke, Belgium

Yuelong Shu, PhD
Director, WHO Collaborating Center for Reference and Research on Influenza
National Influenza Center of China
GENERAL INFORMATION

CURRENCY & BANKING
The currency in Ireland is Euro (€). Banks are open from 10:00 to 16:00 Monday to Friday and there are multiple ATMs available throughout the city and in many stores to make cash withdrawals.

ELECTRICITY
The standard voltage is the Republic of Ireland is 220 VAC at 50Hz. Plugs are 3 pin 1363 type so for European guests an adaptor is required. For US visitors a transformer is required.

INSURANCE
ISIRV, the Local Organizing Committee, and the Royal College of Physicians of Ireland accept no liability for personal injuries sustained or for loss of or damage to property belonging to delegates, either during or as a result of the symposium. Registration Fees do not include insurance. Insurance should be purchased in your country of origin.

REGISTRATION
The fees include the following:
• Participation in the scientific sessions & poster exhibition
• Delegate Pack including Name Badge, Final Programme, Abstracts, Certificate of Attendance
• Welcome Reception, Coffee breaks and lunches

Onsite Registration Desk Opening Hours:

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SHOPPING
Most shops in the city centre, Grafton Street area, open between 09:00 and 10:00 and close at 18:00 Monday, Tuesday, Wednesday, Friday and Saturday. On Thursday most shops offer Late Opening until 20:00. Some shops are open on Sunday from 12:00.

TRAVEL AND TRANSPORT
Airport Taxi: Dublin Airport is 20 minutes from the venue by taxi outside peak hours. The cost is c €20-30 (this is only a guide price so we advise that you confirm the cost with the driver before you start your journey)

Airport Bus- AIRCOACH:
Delegates can use the bus service to Dublin Airport with AIRCOACH.
The Aircoach is a blue bus and the stop closest to the Royal College of Physicians of Ireland is Grafton Street (Trinity College)
For the Return Journey to the Airport the closest stop is Dawson Street (Pink Shirt Shop).
The Aircoach departs every 10 minutes (06:00-19:55) and every 20 minutes at other times.
VISIT DUBLIN APP
We would suggest that while you are in Dublin download the Visit Dublin App which uses technology allowing you to use directional search to move around Dublin and discover your interests. Simply by pointing your Smartphone (iPhone/Android) you will find rich information about the places you see around you. This powerful pointing technology performs directionally filtered searches so that you get the information you are looking for quickly and easily. To find your way to an interesting spot, you can use the convenient Guide Me and/or Camera View augmented reality features.

WEATHER
Dublin weather can be cold in March. Temperatures are usually anywhere between 4°C and 10°C. It is advisable to bring a warm coat and an umbrella.

SOCIAL EVENTS

Wednesday 6th March 2013
WELCOME RECEPTION HOSTED BY LORD MAYOR OF DUBLIN, MR. NAOISE Ó MUIRÍ

The Lord Mayor of Dublin, Mr. Naoise Ó Muirí together with the ISIRV Committee would like to extend a Welcome to the ISIRV symposium delegates at a Welcome Reception being held in their honour in the Oak Room of the Mansion House, Dawson Street, Dublin 2 at 18:00-19:00 on Wednesday 6th March.

Thursday 7th March 2013
EVENING AT JOHNNY FOXES TRADITIONAL IRISH PUB

Meet at 18:30 at the entrance to the symposium venue, The Royal College of Physicians of Ireland, 6 Kil-dare Street. Coaches will depart promptly at 18:45. We will take a scenic route from the centre of the city into the countryside and up the Dublin mountains to the locality of Glencullen. Johnny Foxes pub is nestled in the hills away from the noise of the city. The evening at Johnny Foxes will include Dinner and some Traditional Irish entertainment; music and dancing.

Following the dinner and show the coaches will return to the city centre.
SYMPOSIUM VENUE FLOORPLAN
The Royal College of Physicians of Ireland, Number 6 Kildare Street, Dublin 2

Corrigan and Graves Room Plan
2nd International Symposium on Neglected Influenza Viruses

SCIENTIFIC PROGRAMME

**WEDNESDAY 6TH MARCH**

14.00 - 18.00  **Registration**  Foyer, The Royal College of Physicians of Ireland, Number 6 Kildare Street, Dublin 2.

18.00 - 19.00  **Welcome Reception**  Oak Room, Mansion House, Dawson Street, Dublin 2.

**THURSDAY 7TH MARCH**

08.00 - 08.15  **Welcome and Opening Remarks**  Corrigan Hall
   Ann Cullinane
   Debra Elton

08.15 - 09.00  **Keynote**  “Zooties” of the past and present: what are animal influenza outbreaks trying to tell us about human influenza?
   David Morens MD, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

09.00 - 09.45  **Keynote**  Pigs and influenza pandemics: what did we really learn from the 2009 pandemic?.
   Kristien van Reeth DVM PhD, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.

09.45 - 10.20  **Coffee Break**  Graves Hall

**SESSION I: SURVEILLANCE AND DISEASE INVESTIGATION**  Corrigan Hall

**Moderators:** John Mc Cauley and Debra Elton

10.20 - 10.40  The host specific properties of equine and canine influenza viruses.
   Colin Parrish PhD Baker Institute for Animal Health,
   College of Veterinary Medicine, Cornell University, Ithaca, USA.

10.40 – 11.00  Swine influenza in Central and South America
   Ariel Pereda DVM PhD,
   Instituto Nacional de Technologia Agropecuaria, Buenos Aires, Argentina.

11.00- 11.20  The evolution of influenza virus in North American swine.
   Martha Nelson PhD,
   National Institutes of Health, John E. Fogarty International Center, Bethesda, Maryland USA.
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Shown to stimulate a superior humoral immune response to other commercially available ‘flu vaccines. Duvaxyn Plus has also been proven to give the reassurance of cell mediated immunity.

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11.20 - 12.30 SUBMITTED PAPERS

11.20 - 11.35 Co-ordinated surveillance of influenza viruses in European Pigs: Enhanced Virological and Epidemiological analysis from the European surveillance network for influenza in pigs (ESNIP 3).
*Ian Brown MIBiol PhD,*  
Animal Health and Veterinary Laboratories Agency (AHVLA), Weybridge, UK.

*Sarah Gildea PhD, Virology Unit, Irish Equine Centre, Johnstown, Naas, Co. Kildare, Ireland.*

11.50 - 12.05 Spatial and temporal dynamics of influenza A in North American Swine.
*Amy Vincent DVM PhD, Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, Iowa, USA.*

12.05 - 12.20 Surveillance of equine influenza viruses through the RESPE network in France from November 2005 to October 2010.
*Stephane Pronost PhD,*  
Frank Duncombe Laboratory, Animal Health Department, 14053 Caen, France.

12.20 - 14.00 Lunch and Posters  
Graves Hall (Lunch) and Dun Library (Posters)

14.00 - 14.00 SESSION II: VIRUS TRANSMISSION AND CONTROL  
Corrigan Hall

14.00 - 14.20 Simulation of H3N8 canine influenza virus transmission in dog populations using mathematical modeling and effect of different control strategies on transmission outcomes.
*Cynda Crawford DVM PhD,*  
College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA.

14.20 - 14.40 Pathogenicity and transmission of the novel A (H3N2v) influenza virus isolated from humans in experimentally inoculated pigs
*Amy Vincent DVM PhD,*  
National Animal Disease Center, USDA-ARS, Ames, Iowa, USA.

14.40 - 15.00 The threat of Equine Influenza to International Equestrian Events.
*Graeme Cooke MA Vet MB MBA MRCVS,*  
Federation Equestre Internationale, Chemin de la Joilette 8, 1006 Lausanne, Switzerland.

15.00 -15.30 SUBMITTED PAPERS  
Corrigan Hall

15.00 – 15.15 Equine Influenza Vaccination - Working towards an Evidence Based Regime.
*Ann Cullinane MVB PhD MRCVS,*  
Irish Equine Centre, Johnstown, Naas, Co. Kildare, Ireland.

15.15 - 15.30 Using reverse genetics to define biological differences between equine and canine influenza viruses.
*Kurtis Feng BSc,*  
Cornell University and J. Craig Venter Institute, USA.

15.30 - 16.00 Coffee Break  
Graves Hall
With you all the way
Taking prevention seriously
16.00 - 17.45 **SUBMITTED PAPERS**  
*Corrigan Hall*

**Moderators:** Nicola Lewis and Kristien van Reeth

16.00 - 16.15 Indirect transmission of influenza A virus in pigs.
*Marie Culhane DVM PhD,*  
College of Veterinary Medicine, University of Minnesota, Saint Paul MN 55108, USA.

16.15 - 16.30 Avian H3N8 Influenza Virus Isolated from Harbour Seals.
*Stacey Schultz-Cherry PhD, Department of Infectious Diseases,*  
St Jude Children’s Research Hospital, Memphis, Tennessee, USA.

16.30 - 16.45 Serological status to swine-origin H3N2 influenza viruses in the human population.
*Yu Qiu DVM MSc, Laboratory of Virology,*  
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium.

16.45 - 17.00 Role of receptor binding as a limiting factor in susceptibility of swine to equine influenza virus.
*Tom Chambers PhD,*  
Gluck Equine Research Center and Veterinary Diagnostic Laboratory, University of Kentucky, Lexington KY 40546 USA.

17.00 - 17.15 Efficacy of commercial inactivated and experimental live-attenuated influenza A virus vaccines against infection and transmission of emerging H3N2 in pigs.
*Amy Vincent DVM PhD,*  
National Animal Disease Center, USDA-ARS, Ames, Iowa, USA.

17.15 - 17.30 Epidemiological features of recurrent influenza virus infections in pig farms
*Gaëlle Simon PhD,*  
Anses, Ploufragan-Plouzané Laboratory, Swine Virology Immunology Unit, Ploufragan, France.

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**FRIDAY 8TH MARCH**

08.00 - 08.15 **Opening Announcements**  
*Corrigan Hall*

Gregory Gray

08.15 - 09.00 **Keynote**  
Adventures in Wildlife Influenza.
*Hon Ip, PhD,*  
National Wildlife Health Centre, Madison, Wisconsin, USA.

09.00 - 12.30 **SESSION III CLINICAL AND EXPERIMENTAL VIROLOGY**  
*Corrigan Hall*

**Moderators:** Stacey Schultz-Cherry and Sabrina Swenson

09.00 - 09.20 Evolution of Equine Influenza Viruses at Different Scales.
*Pablo Murcia, DVM, PhD,*  
MRC University of Glasgow Centre for Virus Research Institute of Infection, Immunity and Inflammation College of Medical, Veterinary and Life Sciences University of Glasgow, UK.

09.20 - 09.40 Quantifying the Antigenic Evolution of Equine and Swine Influenza A Viruses.
*Nicola Lewis, BSc, BVetMed, PhD, MRCVS,*  
Centre for Pathogen Evolution, Department of Zoology, University of Cambridge, UK.

09.40 - 10.15 **Coffee Break**  
*Graves Hall*
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10.15 - 12.30 SUBMITTED PAPERS

Peter Daniels PhD,  
Australian Animal Health Laboratory, PMB 24, Geelong, Australia.

10.30 - 10.45 A reverse genetics approach to vaccine breakdown. Can past outbreaks inform surveillance & vaccine strain selection for equine influenza?  
Adam Rash Bsc  
Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, UK.

10.45 - 11.00 Detection of airborne swine influenza A under field conditions.  
Marie Culhane DVM PhD, College of Veterinary Medicine, University of Minnesota, Saint Paul MN 55108, USA.

11.00 - 11.15 The genetics of virus particle shape in equine influenza A virus.  
Paul Digard PhD,  
The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, UK.

11.15 - 11.30 Comparative pathogenesis of Influenza A virus subtypes; pandemic H1N1 2009 and contemporary swine H3N2, in pigs and ferrets.  
Sharon Brookes PhD,  
Animal Health and Veterinary Laboratories Agency, New Haw, Surrey, UK.

11.30 - 11.45 Awareness and Practices regarding Zoonotic Influenza.  
Peter Rabinowitz, MD, MPH,  
Yale School of Medicine, 153 College St, New Haven, CT 06510, USA.

11.45 - 12.00 Morphometry for swine influenza pathogenesis studies.  
Susan Detmer DVM PhD,  
Western College of Veterinary Medicine, Saskatoon, SK S7N 5B4, Canada.

12.00 - 12.15 The effect of demographic characteristics on the humoral immune response elicited by influenza vaccination in donkeys.  
Janet Daly PhD,  
School of Veterinary Medicine and Science, University of Nottingham, UK.

12.15 - 12.30 Antigenic and genetic characterization of swine influenza virus (SIV) isolates from Ohio agricultural fairs.  
Xiu-Feng (Henry) Wan PhD DVM,  
Mississippi State University, Mississippi, USA.

12.30 - 14.00 Lunch and Posters

14.00 - 14.20 SESSION IV EMERGING ISSUES AND NEW DEVELOPMENTS

Moderators: Marie Culhane and Paul Digard

14.00 - 14.20 Salmon anaemia in Chile.  
Marcelo Cortez-San Martín PhD, Centro de Biotecnología Acuícola, Facultad de Quimica y Biolgogía, Universidad de Santiago de Chile, Santiago, Chile.
14.20 - 14.40 One Health A Promising Approach to Neglected Influenza Problems.  
**Gregory C. Gray, MD, MPH, FIDSA,**  
College of Public Health and Health Professions, University of Florida, USA.

14.40 - 15.40 **SUBMITTED PAPERS** Corrigan Hall

14.40 - 14.55 Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses.  
**Ben Hause MS,**  
Newport Laboratories, Worthington, Minnesota, USA.

**Erik Karlsson PhD,** Department of Infectious Diseases,  
St Jude Children's Research Hospital, Memphis, Tenessee, USA.

15.10 - 15.25 Isolation and characterization of a novel bovine influenza C virus from a clinical case.  
**Siao Kun (Jenny) Welch DVM PhD,** VMRD  
Zoetis (formerly Pfizer Animal Health), 333 Portage Road, Kalamazoo MI 49007 USA.

**Elena Govorkova MD PhD,**  
St. Jude Children's Research Hospital, Memphis, Tennessee, USA.

15.40 - 16.15 **Expert Panel** Corrigan Hall

16.15 - 16.30 **Meeting Summary and Close**
2\textsuperscript{nd} International Symposium on Neglected Influenza Viruses
The Symposium Co-Chairs and Scientific Committee are most grateful to

Dr. Stacey Schultz-Cherry
Associate Member, Department of Infectious Diseases
St. Jude Children’s Research Hospital
and
the St. Jude’s IT Team

for their hard work and assistance
in receiving and compiling the abstracts
for this 2\textsuperscript{nd} International Symposium on Neglected Influenza Viruses
THURSDAY 7TH MARCH SESSIONS

SESSION SPEAKER ABSTRACTS & ORAL PRESENTATIONS
Influenza infections of domestic animals have been recognized for at least 350 if not over 400 years, but until recently there has been little interest in studying the combined epidemiology/epizootiology of influenza or of examining influenza in the entire ecosystem in which the virus circulates. To do so may be important because a significant property of influenza viruses is their ability to “host switch”, and there is mounting evidence (e.g., the appearance of the 2009 pandemic virus, and host-switching of different human influenza viruses into pigs) that more than one host may be involved in viral maintenance and evolution, and that such ecosystem complexity may be associated with viral persistence. Several modern and historically important epizootics and epidemics/pandemics will be examined, going back to the pandemic of 1580, and also examining the separate avian and swine epizootics of 1872, evidence for swine epizootics in association with the 1889 pandemic, the 1976 “swine flu affair”, and several aspects of the current situation. A provisional case will be suggested that it may be counterproductive to think of influenza viruses as being host-specific, and that the remarkable ability of the virus to mutate and evolve in a highly complex ecosystem, involving multiple avian and mammalian species, and perhaps other species, has implications for not only influenza preparedness, but also for research aimed at understanding the behavior of influenza viruses in the entire ecosystem of their maintenance and circulation.

KEYNOTE

‘Zooties’ of the past and present: what are animal influenza outbreaks trying to tell us about human influenza?  
David M. Morens, MD  
National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.

Influenza infections of domestic animals have been recognized for at least 350 if not over 400 years, but until recently there has been little interest in studying the combined epidemiology/epizootiology of influenza or of examining influenza in the entire ecosystem in which the virus circulates. To do so may be important because a significant property of influenza viruses is their ability to “host switch”, and there is mounting evidence (e.g., the appearance of the 2009 pandemic virus, and host-switching of different human influenza viruses into pigs) that more than one host may be involved in viral maintenance and evolution, and that such ecosystem complexity may be associated with viral persistence. Several modern and historically important epizootics and epidemics/pandemics will be examined, going back to the pandemic of 1580, and also examining the separate avian and swine epizootics of 1872, evidence for swine epizootics in association with the 1889 pandemic, the 1976 “swine flu affair”, and several aspects of the current situation. A provisional case will be suggested that it may be counterproductive to think of influenza viruses as being host-specific, and that the remarkable ability of the virus to mutate and evolve in a highly complex ecosystem, involving multiple avian and mammalian species, and perhaps other species, has implications for not only influenza preparedness, but also for research aimed at understanding the behavior of influenza viruses in the entire ecosystem of their maintenance and circulation.

KEYNOTE

Pandemic Potential Of Swine Influenza Viruses  
Prof. Kristien Van Reeth  
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium

Three animal influenza viruses of the last decade have been extremely interesting from a public health perspective. Since 2003, the highly pathogenic avian H5N1 influenza virus has occasionally infected humans, as well as pigs. But pigs did not play a role in H5N1 virus transmission to humans, and the virus so far failed to become adapted to either animal species. The 2009 pandemic H1N1 influenza virus was a reassortant between established swine influenza viruses from North America and Europe, with gene segments of avian, human and swine origin. However, it remains unknown how, when and where this specific reassortant emerged, and what made it fit for efficient human-to-human transmission. The H3N2 “variant” influenza virus is another recent swine-origin reassortant, which has infected some 300 children in fair settings in North America in 2011 and 2012. It remains to be proven whether the latter virus is more fit for zoonotic infections than other enzootic swine influenza viruses but, unlike the 2009 pandemic H1N1 virus, it did not spread further in the human population. Each of these 3 viruses has reminded us of our ignorance about the exact role of pigs in influenza pandemics. More specifically, we lack insights into what is needed for adaptation of avian influenza viruses to pigs, for influenza virus transmission from pigs to humans, and for sustained transmission of swine-origin viruses in the human population. On the positive side, swine flu researchers have now started to undertake experimental studies to address these questions. In my lecture I will present a critical summary of these timely studies and the major lessons from them.

SESSION I – SURVEILLANCE AND DISEASE INVESTIGATION

The Host Specific Properties Of Equine And Canine Influenza Viruses  
Colin Parrish, J Hayward, B Zhou, D Wentworth, K Feng.  
Cornell University, J Craig Venter Center

The A/H3N8 CIV is a variant of EIV, and emerged around 2000 in dogs. We examined the evolution of the virus in horses and dogs, and showed that the CIV arose from the introduction of a single EIV into dogs. That virus has remained in circulation in dogs in the USA, where it is being maintained in a small number of animal facilities that contain high numbers of dogs, and with many dogs passing through and becoming infected at high rates. Here we examine the evolution of the viruses in dogs, and report the results of examination of the sequences of viruses from high population and throughput facilities, and that showed that a single strain of virus has been circulating for a number of years. The sequences of the viruses have been evolving, and there was a higher rate of sequence variation among the viruses in dogs than was historically seen for the EIVs. We examined the sequences of the viruses after extended passages in dogs, and saw a number of changes that were CIV-specific, and have followed up with those using molecular genetic approaches to define their roles in controlling tropism and host specificity, first examining the specific mutations in HA and NA and their roles in controlling binding to the modified sialic acids of the horse and dog erythrocytes and tissues. Because of the limited maintenance of the CIVs in high density populations of dogs, there is an opportunity to eradicate the viruses completely from dogs. We propose and model a number of approaches that could be considered for this effort, including breaking the transmission cycle, or using an attenuated intranasal vaccine to compete with the wild virus.
Swine Influenza In Central And South America
Ariel Pereda, DVM PhD
Instituto de Virología, Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina

Influenza A viruses (IAV) are important pathogens responsible for economic losses to the swine industry and represent a constant threat to public health. The clinical presentations of IAV infection in naïve swine population are associated with outbreaks of acute respiratory disease in which morbidity can reach up 100%. In Central and South America, reports of Swine Influenza Virus (SIV) are scarce. Information on SIV activity in Latin America pales in comparison with the wealth of knowledge resulting from SIV surveillance studies in North America, Europe and Asia. The evolutionary and antigenic relationships among SIVs in Central and South America need be determined.

In Argentina, the presence of antibodies against SIV has been detected since 1971 in pig operations in Antioquia, showing an overall seroprevalence of 21%. In 2011, a serological survey on 78 herds, from the three major swine rearing areas of Colombia, was carried out. Besides nasal swabs, lungs and bronchial aspirates were processed for virus isolation. Overall serological reactivity by HI test to H1 was 69.0% and H3 49.3%. Fifteen strains belonged 9 of them corresponded to H1N1pdm and 3 to H1N1 classical strains.

In Brazil, a great number of cross-sectional serological studies have indicated the circulation of H1N1, pdmH1N1 and H3 SIV strains in Brazilian pigs. However, no additional information about the isolated strains was presented. During a 2009-2010, an outbreak of mild respiratory disease in growing pigs and sows detected in a pig herd in Santa Catarina State, Brazil. Immunohistochemistry revealed positive staining in the nuclei of the bronchiolar epithelial cells. Lung tissue from piglets and nasal swabs from sows were positive for influenza A by RT-PCR. An Influenza virus was isolated and the sequences of HA and M genes revealed that the virus was homologue with the pdmH1N1. This was the first report of an outbreak of pdmH1N1 influenza virus in pigs in Brazil. In 2011 and 2012 EMBRAPA analyzed 646 samples of nasal swabs or lung tissue collected from pigs of various ages and raised in commercial herds in Southern Brazil. A total of 111 (17.18%) samples were positive by RT-PCR and 46 (41.44%) influenza viruses were isolated. Complete and partial genomic sequences of 25 SIV were obtained. Based on the sequence analyses 16 influenza viruses showed a high identity (98-100%) with pdmH1N1. Five influenza viruses were closely related to an American H3N8 equine influenza virus and four virus isolates revealed to be a novel reassortant H1N2 influenza virus that had not been detected in pigs in Brazil before the recent influenza outbreak in pigs.

The circulation of SIV in pigs in Argentina was initially explored by retrospective serology showing the presence of approximately 41% of positive sera by ELISA. Whole human H3N2, pH1N1 (Argentina reported the second occurrence of the pH1N1 in pigs in the world), and novel SIVs in pigs derived by independent reassortment events were reported in Argentina. After clinical, pathological and virological findings have suggested that the infection was widespread among Argentinean pig farms. The SIVs isolated in Argentina are distinguishable from SIVs in North America and represent independent transmission events. At this stage, it is not known whether reassortment among SIVs in Argentina is a common occurrence and/or reflect the exponential growth of the swine industry in the region. In Venezuela, in 2004 a limited serological survey against SIV subtypes H1 and H3 showed 7.9% and 8.2% of positive sera in 7 out of 10 of the farms studied. Further, a cross-sectional serological study in four farms along a year showed concurrent infection with H1 and H3 SIV subtypes with higher seroprevalence of H3 during the raining season (from May to October), with a cyclic pattern during the study period.

In Guatemala a two-year (2010-2011) cross-sectional study for the detection of pH1N1 virus and other SIVs circulating in pig populations was carried out. Influenza virus infection was detected by RRT-PCR (15.8%) and by serological testing (10.2%). A higher proportion of RRT-PCR virus positive samples were observed in herds from commercial farms in comparison to backyard populations. Spatial analyses suggested that positive farms tended to cluster yearly; two clusters were observed in 2010 and one cluster in 2011. These clusters were located 1) on the border between Guatemala and Honduras, 2) around the capital, Guatemala City, and 3) in departments where several influenza viruses have been confirmed in humans.

The knowledge of the circulating strains in the region will make it possible to be able to design better diagnostic tests and immunogenicity, due to the very low homology at the amino acid level of the viral proteins between the different strains of Influenza virus. It is for this reason that it is essential to carry out an epidemiological surveillance of the SIV and recognize their molecular, antigenic and pathogenic characteristics of these viruses. Also it is important to state that instead of the potential direct impact of this disease in terms of public health, it is also important to understand another impact that this disease has in Public Health. There are several infections associated with SIV and an increase in the use of antimicrobial agents to control secondary infections increase the risk of bacteria resistance to various antibiotics and increased residues in pork.

The Evolution Of Influenza Virus In North American Swine
Martha Nelson PhD
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The evolution of genetically diverse influenza viruses in North American swine represents an important pandemic threat, as recently demonstrated by the emergence of the 2009 pandemic H1N1 virus (H1N1pdm09) in the Americas and the infection of >300 humans with the swine-origin H3N2v virus in the United States since July 2011. Recent increases in genetic sequencing of influenza viruses in North American swine have revealed the importance of three key processes in the evolution of genetic diversity within the swine host: (a) human-to-swine transmission, (b) frequent genomic reassortment, and (c) spatial dissemination of viruses following routes of swine transport. Here I describe how frequent human-to-swine transmission continually introduces genetically novel
viruses into the swine population, the patterns by which these viruses then reassort with previously circulating swine viruses, and how long distance transport of swine spreads genetic diversity regionally. In particular, I will explain the roles of human-to-swine transmission, reassortment, and spatial spread in the recent evolution of the H3N2v viruses, including their associated pandemic matrix (MP) segment. Recognizing the roles of these key processes in the evolution of genetic diversity is central to understanding how novel viruses emerge in swine, including those with pandemic potential, and identifies new targets for control and surveillance.

**Coordinated Surveillance Of Influenza Viruses In European Pigs: Enhanced Virological And Epidemiological Analysis From The European Surveillance Network For Influenza In Pigs (Esnip3)**

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The human influenza pandemic H1N1 2009 virus has subsequently spread around the world infecting pigs. Although the virus did not emerge in pigs in Europe there were gaps in surveillance for influenza in pigs that caused concern for global veterinary and public health officials. As a result the EU funded a European network to address the epidemiology, identification and detailed characterization of influenza viruses circulating in European pig populations. To develop programmes of harmonized surveillance across Europe and coordinate appropriate identification and characterization of viruses. This will include collecting epidemiological data to understand distribution and spread of the virus, basic virological characterization of virus subtypes identified, antigenic cartography on selected strains, and full genome genetic characterization. An extensive virological surveillance system has been harmonised and established in 14 countries. Influenza virus has been detected in 1034/3407 herds examined (30% positive) from which 675 strains were sequenced. All viruses were first generation reassortments from pdm09 strains and several co-circulating strains. The unique emergence of first generation reassortments from pdm09 strains and preliminary antigenic maps which will be informative for vaccine strain selection have been demonstrated for the first time. The potential emergence of some of these strains may have implications for zoonotic infection in the future.

**Epidemiological And Virological Investigation Of Equine Influenza Outbreaks In Ireland (2010 – 2012)**

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Between November 2010 and October 2012 equine influenza outbreaks were diagnosed on nine premises in five of the 32 counties of Ireland. Outbreaks occurred in a variety of animals including Thoroughbred horses, non-Thorouhbred horses, ponies and donkeys. Premises included training yards, a stud farm, an artificial insemination station, a third level equestrian college, dealers yards and private farms. Vaccination and clinical histories were obtained from the attending veterinarian clinician and personnel involved in the day to day management of the horses. The premises were visited, nasal swabs and blood samples were collected and extensive virological investigations undertaken. The aims of the study were to identify (a) the factors involved in the spread of equine influenza in a partially immune population, (b) the most effective laboratory tests for diagnosis and epidemiological investigation (c) the virus strains responsible for the outbreaks (d) evidence of vaccination breakdown. All outbreaks were associated with movement of horses but there was no epidemiological link between premises. Veterinary advice was sought on average more than four days after the first clinical signs were observed. Clinical signs were observed in 15-80% of horses with young horses being most severely affected. Virus spread was limited within affected premise. On five premises analysed to-date where samples were collected on at least three occasions, equine influenza virus infection was confirmed by laboratory testing in 56% of the horses. Initial diagnosis on all premises was made by RT-PCR. On five premises analysed fewer positive horses (14.8%) were identified using a Light Cycler SYBR Green based assay than by a Taqman primer probe assay (24.4%). Similarly fewer seroconversions were identified using the Haemagglutination Inhibition test (42%) compared to the Single Radial Haemolysis test (50%). To-date the haemagglutinin gene of viruses from eight of the nine affected premises has been sequenced. All viruses belong to Clade 2 of the Florida sublineage. None of the vaccines have been updated with a Clade 2 virus in line with the current OIE recommendations. Vaccination failure was observed with three vaccines available on the Irish market during the study period.
Repeated and rapid spread of influenza A viruses (IAV) challenges us with preparing for the next epidemic or pandemic. One strategy for preparedness is increasing our knowledge of IAV in animal populations that may be a risk to humans. It is essential for both animal and human health to gather meaningful data, particularly concerning changes that may increase transmissibility and/or virulence. This is reliant upon timely epidemiological, phylogenetic, and virological analyses. To better understand IAV in the swine population, we undertook a phylogenetic analysis of 1004 HA, 965 NA and 973 M sequences of IAV identified from swine during 2009 – 2012 in voluntary and anonymous submissions into the USDA IAV swine surveillance system. Analyses revealed that multiple clades of A/H1N1, A/H3N2, and A/H1N2 co-circulated in the United States during this period. We further categorized viral isolates to one of seven genetically and antigenically distinct hemagglutinin lineages (H1a, H1b, H1y, H1s1, H1s2, H1N1pdm09 and H3 cluster IV). There was a dramatic increase in the occurrence of H1s1 in samples submitted with a concomitant decrease in H1N1pdm09 since 2009. H3 cluster-IV demonstrated considerable diversity, with emergence of at least 10 unique phylogenetic clades. Although the H3N2 represented less than 30% of the identified viruses during the total time period, this subtype represented an increasing proportion of sequenced isolates since late 2011. We further analyzed patterns of spatial diversity using autoregressive integrated moving average models that identified absolute humidity, mean air temperature, and estimates of swine population density as the best predictors of co-circulating viral diversity. In conclusion, substantial progress has been made since 2009 to improve the surveillance of IAV in swine in the United States. The number of sequences has increased dramatically, allowing a better understanding of genetic diversity along with seasonal and temporal patterns. These data allow for the identification of better intervention strategies such as vaccine and diagnostic updates, as well as providing insight into determinants of transmission that could be mitigated by changes in production practices or facility management.

Surveillance Of Equine Influenza Viruses Through The Respe Network In France From November 2005 To October 2010

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Outbreaks of equine influenza (EI) in endemic population cause economic loss despite a greater surveillance and vaccination. According to the Federation Equestre Internationale (FEI), all horses attending to their competitions have to be vaccinated in the previous 6 months. Vaccination reduces symptoms and limits virus propagation, but doesn’t completely protect against new infection. Therefore, equine influenza virus (EIV) surveillance is essential as an early warning system to veterinary practitioners, professionals within the equine industry and the concerned institutions. RESPE (French epidemiological network for equine diseases) is the first European network for epidemi-surveillance of major equine diseases based around sentry veterinarians in France. The aim of this present study was to demonstrate how the RESPE had contributed to a more efficient surveillance of EI in France since 2006. 920 nasopharyngeal swabs sent by RESPE-associated veterinarians were analysed. 582 respiratory samples were sent by non RESPE-associated veterinarians. RNA was extracted from 140 µL of respiratory fluids with the QiAamp Viral RNA minikit. Detection was performed by rRT-PCR amplification targeting the M1 gene. Phylogenetic analysis was performed after a RT-PCR amplification targeting the H1 fragment of hemagglutinin (H3) gene, directly on RNA extracted from nasal swab. Among 920 samples sent by RESPE-associated veterinarians, 121 (13.1%) were positive for EIV divided into 42 premises. Horses infected by EIV were mainly those participating to equestrian manifestations with a majority of French trotters (44.6%), French saddle ponies (23.1%) and ponies (9.9%). Among the 582 samples received from non RESPE-associated veterinarians, 26 (5.1%) EIV positive horses were detected. The repartition of EIV positive cases is clearly related to the localization of the RESPE veterinarians. The last important outbreak was observed between February and May 2009. 70 horses were found positive by rRT-PCR. Fifteen of the 23 premises, including the Grosbois training yard (index premise), were managed by RESPE-associated veterinarians. The HA1 nucleotide sequence was completely determined on 39 strains and partially on 8 others. All strains analyzed in this study belonged to the American lineage, Floridian sublineage. Clade 1 and Clade 2. Clade 1 was identified only during the Grosbois episode. The findings of this study demonstrate that the analysis realized by the RESPE allowed detecting more EIV in France. RESPE also allow characterization of the strains and participate to epidemiological and vaccine efficacy survey. Implementation of this type of network in other countries should reduce the economic losses associated with outbreaks of EI.
SESSION II – VIRUS TRANSMISSION AND CONTROL

Simulation Of H3n8 Canine Influenza Virus Transmission In Dog Populations Using Mathematical Modeling And Effect Of Different Control Strategies On Transmission Outcomes

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Dogs in high density/high turnover communal settings such as animal shelters and boarding kennels are at highest risk for exposure to canine influenza A subtype H3N8 (H3N8 CIV). Viral and population parameters that influence H3N8 CIV transmission in communal settings have not been defined. Knowledge of the role of these factors in promoting virus transmission is critical to development of intervention strategies. The objectives of this study were: 1) to develop a mathematical model for H3N8 CIV transmission in animal shelters to assess the importance of multiple viral and population factors in spread of virus between dogs; and, 2) to apply 3 common intervention strategies to the model to determine their efficacy in reducing or eliminating virus transmission. A deterministic model was developed where dogs were divided into 5 classes based on known biological properties of H3N8 CIV: susceptible, latent (time from virus exposure to virus shedding), clinical infection, subclinical infection, and recovered. Model parameters accounted for population size, dog transitions between the 5 classes, and direct and indirect (fomite) virus transmission factors. For model simulations, a single latently infected dog was introduced into shelters divided into 5 classes, and direct and indirect (fomite) virus transmission factors. For model simulations, a single latently infected dog was introduced into shelters containing from 50 to 400 dogs and 500 simulations were conducted with varying ranges for all model parameters. The following outcomes were analyzed: occurrence of an epidemic, epidemic peak and duration, number of infected dogs, establishment of endemic infection, and virus transmission. Isolation of sick dogs and vaccination in the shelter prior to virus introduction, and quarantine. H3N8 CIV epidemics occurred in 99.6% (498/500) of the simulations. Endemic infection was established in every case. Large numbers of infected dogs accumulated during the epidemics due to entry of more susceptible dogs on a daily basis. Most epidemics were primarily due to indirect virus transmission. Isolation of sick dogs and vaccination were not effective strategies for prevention of epidemics and endemic infection. Quarantine was partially effective in that it did not stop epidemics but prevented endemic infections due to virus die-out. This is the first study to model H3N8 CIV transmission in a dog population. A unique feature of this model was the inclusion of both direct and indirect virus transmission. Indirect virus transmission and the number of dogs in the population have the greatest impact on H3N8 CIV transmission dynamics.

Pathogenicity And Transmission Of The Novel A (H3n2v) Influenza Virus Isolated From Humans In Experimentally Inoculated Pigs

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Human cases with H3N2 (H3N2v) viruses closely related to swine H3N2 viruses were detected in 2011 and increased to >320 cases by the end of 2012. H3N2-TRIG was the H3N2 genotype endemicly circulating in the U.S. swine population prior to the emergence of H1N1pdm09, and H3N2p are novel H1N1pdm09/H3N2-TRIG reassortant genotypes. Whole genome analysis showed ten different H3N2p genotypes present in the U.S. swine population since 2009. Genotype 1 (G1) acquired the pM gene alone from H1N1pdm09 similar to H3N2v, and was most frequently detected among H3N2p in pigs. This indicates a genetic fitness of G1 in swine and possibly transmission to human. Hemagglutinin (HA) gene analysis indicated that rH3N2p and H3N2v are related to the contemporary H3N2-TRIG virus, but recent rH3N2p swine isolates formed separate clusters. We compared the pathogenic, transmission, genetic, and antigenic properties of a human H3N2v isolate with two swine H3N2 viruses, H3N2-TRIG and rH3N2p in the swine host. Groups were assigned as negative controls; H3N2v-infected; H3N2-TRIG-infected and rH3N2p-infected, with contact pigs in each group to study virus transmission. No differences in pathogenicity or transmissibility among the three H3N2 viruses were detected. Antibodies to H3N2-TRIG virus cross-reacted to H3N2v. However mutations at putative HA antigenic sites of contemporary swine H3N2 viruses were detected with reduced serologic cross-reactivity among the rH3N2p sub-clusters. The findings demonstrate antigenic drift of these new viruses concurrent with the genomic reassortment. As influenza A viruses continue to evolve and transmit between humans and animals, the role of pigs in generating reassortant influenza viruses cannot be overlooked. Continued genetic, antigenic, and in vivo studies in swine are essential to determine the phenotypes of these novel emerging viruses in their natural host.
The Federation Equestre Internationale (FEI) is the world governing body of equestrian sport, providing not only the regulations ensuring fairness on the field of play at the international, but crucially also provides the regulations of a veterinary nature which protect and manage horses within the elite levels of the sport. The regulations are wide ranging, from the standards of facilities provided for horses, biosecurity measures e.g. vaccinations, welfare measures, methods of identification, processes that ensure that horses that are not fit to compete do not, anti-doping measures, and the roles and tasks of the many veterinarians required to conduct the sport. The FEI answers to 134 National Federations. The number of international FEI events has astonishingly almost doubled over the last 6 years and reflects socio-economic changes in equestrian sport, that need urgent attention in order to ensure better protection against the threat of disruption.

Equine Influenza Vaccination – Working Towards An Evidence Based Regime
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Despite the implementation of mandatory vaccination programs in racehorses, equine influenza outbreaks continue to occur amongst this highly mobile population. There is no international harmonisation of vaccination requirements but many racing authorities require that horses receive two primary vaccinations administered 21-92 days apart followed by a third vaccination administered 150-215 days after the second dose and annual vaccination thereafter. This regime is neither evidence based nor has it has been systematically evaluated. The object of this study was to determine the effect of varying vaccination intervals in accordance with the mandatory programme.

Fifty seven unvaccinated Thoroughbred horses on four farms were vaccinated with a subunit ISCOM based vaccine (Equip FT). All horses in this study (10 weanlings, 17 yearlings, 14 two year olds and 16 three year olds) were seronegative for equine influenza at the time of first vaccination. Within each age group they were randomly allocated one of three primary vaccination regimes. R1 received their two primary vaccinations three weeks apart and their third vaccination (V3) five months after the second dose (V2) i.e. at the shortest intervals permitted by the racing authorities. R2 were vaccinated in accordance with the manufacturers recommendations i.e. they received their two primary vaccinations six weeks apart and V3 five months after V2. R3 received their two primary vaccinations thirteen weeks apart and V3 seven months after V2 i.e. at the longest intervals permitted by the authorities.

The antibody response of all horses was monitored by Single Radial Haemolysis for 11 months i.e. up to three weeks after R3 received V3. The mean antibody response to V2 and the subsequent rate of antibody decline was similar in all three groups. Analysis of the response to V3 is in progress. The periods between vaccine doses when the mean antibody level was below that consistent with protection, were far greater for R3 than for the other two groups. Thus, lengthening the vaccination intervals of the primary course did not appear to inhibit the response to vaccination but increased the immunity gap. The factors that influenced the variable response of individual horses to vaccination will be explored. The practical significance of the study findings for the management of racehorses.
and their potential contribution towards the international harmonisation of vaccination regulations will be discussed.

**Revealing Tropism Differences Between EIV And CIV**

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Canine influenza virus (CIV) emerged in 2004 in racing greyhounds in Florida, USA. The virus arose from a direct transmission of equine influenza virus H3N8 (EIV) into dogs. Since then, CIV has been primarily circulating in animal shelters where there are high density populations and rapid turnover rates of dogs. Biological differences between EIV and CIV are poorly understood. Thus, we sought to uncover and understand any tropism differences between the two viruses. Eight-plasmid influenza reverse genetics systems were optimized to rescue wildtype EIV between the two viruses. Eight-plasmid influenza reverse genetics systems were optimized to rescue wildtype EIV between EIV and CIV. We anticipate there will be clear biological differences in the HA2 region, and adjacent to the sialic acid binding site. Differences in the cleavage site, the glycosylation site in occur between the HA genes, and we are examining the greatest number of amino acid residue differences that differed in infection efficiency due to host-range barriers. Single and double reassortments of HA and NA are being sought to uncover and understand any tropism differences between EIV and CIV.

**RESULTS:** In group I, all experimentally inoculated pigs (8/8) were IAV infected and all direct contact controls were infected (2/2) following direct exposure to infected pigs. IAV positive group 1 pigs increased from 5 to 10 over time. IAV was detected on a low proportion of fomite samples. Eleven of 144 fomite samples (8%) were low level RRT-PCR positives (Ct value >35 and <40). One replicate of each sentinel groups LB and HB became IAV infected. In each of these replicates, all five pigs were infected over time and confirmed positive by RRT-PCR. All pigs in group NC and the remaining replicates of sentinel groups LB and HB remained negative.

**DISCUSSION:** This study provides evidence of indirect transmission of IAV from infected to sentinel pigs. Transmission occurred in two replicates under differing biosecurity settings. High biosecurity procedures were not able to fully prevent transmission. Further work is in progress to help identify the most likely gap which led to transmission in the HB group.

**Avian H3N8 Influenza Virus Isolated From Harbor Seals Transmits In Ferrets**

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The recent isolation of a unique, wholly avian-derived H3N8 influenza virus from New England harbor seals that has naturally acquired mutations previously shown to increase the virulence and transmissibility of highly pathogenic avian H5N1 influenza viruses in mammals has generated a great deal of scientific interest. To elucidate the potential human health threat, we evaluated the virulence of a panel of avian and mammalian H3N8 viruses in vitro and in vivo including a ferret model of virus transmission. Our studies demonstrate that the seal H3N8 virus replicates in human lung cells and causes more severe disease in mice as compared to the other viruses, including other avian, canine and equine viruses. More importantly, it readily transmits via direct contact but not by respiratory droplets in ferrets suggesting that these avian-derived H3N8 viruses warrant closer examination and enhanced surveillance to monitor potential risks to human
health as they exhibit a number of phenotypic traits associated with mammalian adaptation.

Cross-Reactive Antibodies To European H3N2 Swine Influenza Virus In Humans

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Objectives
H3N2 influenza viruses of humans and swine are derived from the pandemic A/Hong Kong/68 virus, but they have followed different evolutionary pathways over time. The H3 of current European H3N2 swine influenza viruses (SIVs) is antigenically and genetically most closely related to human viruses from the 1970s, and most distinct from contemporary human H3N2 viruses. This suggests that young people in particular may lack cross-reactive antibodies to European H3N2 SIVs.

Methods
To test the above hypothesis, we have performed hemagglutination-inhibition (HI) assays on 80 sera from people born between 1913 and 2007. The sera had been collected from people in The Netherlands in 2008. They were tested against an old human H3N2 virus A/Victoria/3/75, a more recent human H3N2 virus A/Wisconsin/67/05, and the European H3N2 SIV sw/Gent/172/08.

Results
Antibodies to A/Victoria/3/75 were found in 87% of the persons born before 1980, and in only 3% of the persons born after 1980. Conversely, antibodies to A/Wisconsin/67/05 were found predominantly in the latter age category (86% positives), whereas 42% of the people born before 1980 tested positive. Antibodies to sw/Gent/172/08 were almost exclusively found in people born before 1980, with 89% seropositives. The presence of antibodies to the H3N2 SIV was strongly correlated with seropositivity to A/Victoria/3/75.

Conclusions
These preliminary data indicate that people born after 1980 in particular may lack immune protection against European H3N2 SIVs.

Role Of Receptor Binding As A Limiting Factor In Susceptibility Of Swine To Equine Influenza Virus

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As part of our studies exploring the possible role of the horse in interspecies transmission of influenza viruses, we performed in vivo infection experiments on mini-pigs with the objective of determining whether swine were susceptible to infection with any of a series of mutant equine H3N8 influenza viruses bearing human H3 HA. These mutants differed in their receptor binding properties and were described in Suzuki et al., J. Virol. 74, 11825, 2000. Wild-type equine H3N8 and swine H3N2 viruses were used as controls. By real-time RT-PCR, virus-positive nasal swabs were produced from at least 1 pig infected with each virus although quantitatively much reduced compared to wt swine influenza. Histopathologically, all viruses except wt equine influenza produced moderate to severe tracheal inflammation. Only wt swine influenza induced fevers. Since swine are expected to be susceptible to human influenza virus bearing the mutant HAs, these results suggest that other viral functions besides HA receptor binding are important factors limiting equine H3N8 influenza virus infection of swine. In other work, we identified several avian influenza viruses capable of replication in explanted equine tracheal epithelium. One such virus, subtype H1N2, was also associated with enhanced nasal shedding. No clinical signs, virus shedding, or seroconversion were detected. This virus is evidently not infectious for equines even with heavy exposure, which emphasizes the limitations of extrapolating from the equine tracheal explant system to actual equines.

Efficacy Of Commercial Inactivated And Experimental Live-Attenuated Influenza A Virus Vaccines Against Infection And Transmission Of Emerging H3n2 In Pigs

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The objective of this study was to evaluate the ability of commercially available inactivated swine influenza A virus (IAV) vaccines licensed in the USA to protect against primary infection with a swine H3N2 reassortant virus (H3N2p) containing the H1N1pdm09 matrix gene. The whole genome of the rH3N2p is similar to the H3N2v that infected humans in the USA in 2012. The effectiveness of the vaccines in preventing aerosol transmission from primary infected pigs to naïve pigs in an indirect contact model was assessed. We also evaluated the efficacy of two experimental live-attenuated influenza virus (LAIV) vaccines in the same manner. One commercial vaccine provided significant partial protection, as viral titers were reduced when compared to non-vaccinated pigs, whereas the other two licensed vaccines provided limited efficacy. However, none of the commercial vaccines prevented transmission to naïve contacts, as all indirect contact pigs seroconverted, indicating that reduction in nasal shedding of the principal pigs was insufficient to prevent aerosal transmission. LAIV-2, with a more contemporary H3 than LAIV-1, provided complete protection from challenge, as virus was not recovered from nasal swab or lung lavage from any primary challenged pig and none of the indirect contact pigs seroconverted. We did not observe clinical disease or fever in any of the pigs, even those that were not vaccinated, which is a consistent observation in pigs experimentally infected with this genotype of H3N2p swine viruses. Thus, clinical presentation alone is unlikely to be definitive in determining if pigs are infected. Minimal lung pathology was observed in non-vaccinated challenge control pigs, thus, reduction in lung lesions in vaccines
was not a conclusive measure of protection. While the commercially available swine IAV vaccines we evaluated may reduce viral shedding, none were able to prevent indirect transmission of the rH3N2p challenge virus to naïve pigs. However, an LAIV encoding a contemporary H3 protein provided complete protection and thus, eliminated transmission to naïve pigs. This has important implications for virus control on farm as well as at points of concentration of pigs of mixed immune status at the human-animal interface such as agricultural fairs or markets.

### Epidemiological Features Of Recurrent Influenza Virus Infections In Pig Farms

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Repeated infections by different Influenza A virus subtypes within pig farms increase the risk of emergence of novel reassortant viruses. The objectives of this study were to deeply characterise the epidemiology of recurrent infections with swine influenza viruses, for a better understanding and identification of main determinants of this particular epidemiological form of the flu disease. A longitudinal study was carried out in 3 selected pig farms located in Brittany, France, and known for being affected by recurrent influenza infections for several months. Three batches were followed within each farm, from piglets' birth to slaughter, through a representative sample of 40 piglets per batch. Piglets were monitored individually on a monthly basis for serology and daily for clinical and virological investigations when a flu outbreak occurred, during the whole clinical outbreak. Influenza outbreaks, confirmed by Influenza A virus detection, were observed at least once in each followed batch. These outbreaks occurred at constant age within farms, most often in nursery and were correlated with an increase in the frequency of sneezing and coughing fits. Avian-like swine H1N1 and human-like reassortant swine H1N2 viruses from European enzootic lineages, as well as reassortant viruses generated from these parental enzootic viruses, were identified consecutively and sometimes simultaneously according to studied batches. These concomitant detections suggested co-circulation of different viruses at the farm, batch and sometimes individual levels. A great variability was observed between farms in terms of dynamics of infection and humoral immune responses. These two latter components were strongly influenced by rearing conditions, age at infection, and the level of passive immunity transmitted by the dam.
Notes
NEGLECTED INFLUENZA MEETING

FRIDAY 8TH MARCH SESSIONS

SESSION SPEAKER ABSTRACTS & ORAL PRESENTATIONS
and infect mammals. and only a few (or none?) additional changes are contain mutations necessary for mammalian-adaptation, transmission of the seal virus via direct contact. We have been unable to show aerosol-mediated transmission in the laboratory, infected ferrets are capable of weight loss in infected BALB./C mice. Finally, while we further evidence of mammalian adaptation, the seal virus, including those from dogs and horses. Moreover, as mammalian cell lines was faster than other H3N8 viruses, replication of the seal virus in MDCK and other possible roles of these differences and found that the initial virus from its avian counterparts. Erik Karlsson and Stacey Schultz-Cherry have collaborated with us to investigate the origin, most closely related to waterfowl viruses from the Atlantic and Midwestern United States. A number of sequences changes were noted that differentiated the seal virus from its avian counterparts. Erik Karlsson and Stacey Schultz-Cherry have collaborated with us to investigate the possible roles of these differences and found that the initial replication of the seal virus in MDCK and other mammalian cell lines was faster than other H3N8 viruses, including those from dogs and horses. Moreover, as further evidence of mammalian adaptation, the seal virus, unlike contemporary avian H3N8 viruses, is able to induce weight loss in infected BALB./C mice. Finally, while we have been unable to show aerosol-mediated transmission in the laboratory, infected ferrets are capable of transmission of the seal virus via direct contact. We hypothesize that some wild bird influenza viruses already contain mutations necessary for mammalian-adaptation, and only a few (or none?) additional changes are necessary before the virus can cross the species barrier and infect mammals.

SESSION III – CLINICAL AND EXPERIMENTAL VIROLOGY

Evolution Of Equine Influenza Viruses At Different Scales

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Influenza A viruses (IAVs) are significant pathogens of humans and animals that constitute a threat to public health and food security. Despite the vast body of knowledge on influenza biology we still don’t know the underpinning mechanisms of key issues such as antigenic variation and cross-species jumps. Understanding the mechanisms that shape viral phylogenies at different scales (from the individual to the population) is key to understand such issues and to design appropriate intervention measures to combat infectious diseases.

Equine influenza virus (EIV, H3N8) is a significant pathogen of the horse that has been circulating globally for around 50 years. Like human influenza viruses, vaccines against EIV need to be regularly updated due to antigenic drift. Moreover, in early 2000’s EIV jumped the species barrier and established as a respiratory pathogen of the dog, canine influenza virus (CIV). Thus, EIV is a unique natural model system to study antigenic variation and viral emergence.

I will discuss the evolutionary dynamics of EIV at the intra- and inter-host level in naïve and previously vaccinated horses under experimental conditions, link it with observed data at the regional and global scale and highlight their epidemiological implications.

Using Antigenic Cartography To Quantify Antigenic Evolution In Swine And Equine Influenza A Viruses And For Vaccine Strain Selection

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Swine and horses are important hosts for influenza A viruses where outbreaks of disease have significant economic impact. Virus variants arising in either pigs or horses could have zoonotic potential for other host species including humans, with the potential to cause epidemics and pandemics. The haemagglutinin (HA) protein is of key importance in the control of swine and equine influenza because HA is the primary target of the protective immune response and the main component of currently licensed influenza A vaccines. Since the influenza A virus HA protein changes over time via a process called antigenic drift, vaccine strains must reflect the currently circulating strains to remain effective. Vaccinating pigs against influenza A virus with fully licensed commercial vaccines or autogenous vaccines is a common practice in the U.S. swine industry. Autogenous vaccine usage against influenza A virus has continued to increase due to the greater diversity of viruses co-circulating in the North American pig population and the inability of the animal biologics industry to change the vaccine composition as
rapidly as the viruses are changing. Conversely, swine influenza A virus vaccine use is more limited in EU countries but varies among the member states. There is a marked heterogeneity among currently circulating H1 and H3 strains in swine in North America and the EU and there is no formalized method of vaccine strain selection. In horses, only one subtype – H3- currently circulates and the OIE expert surveillance panel for equine influenza annually reviews the strains used in the equine influenza virus vaccine by considering antigenic, genetic, and epidemiological data on currently circulating strains with provision of reference reagents for quality assurance. For equine influenza H3 we have shown that just one key amino acid substitution can result in antigenic variation that would result in a loss of vaccine protection. Furthermore, equine vaccine cross-protection studies have shown that vaccination with a strain from one cluster does not prevent infection with a strain from the other co-circulating cluster. Antigenic drift is assessed primarily by the haemagglutination inhibition (HI) assay. HI assay data can be more thoroughly analyzed by using additional visual and computational methods such as antigenic cartography. Using antigenic cartography, we quantified and visualized the antigenic differences among viruses as an antigenic map. By integrating these measured antigenic differences with the genetic sequence data and experimental cross-protection studies in relevant hosts, we have an effective tool to study and understand the molecular basis of antigenic evolution in both pigs and horses. These maps are accordingly used as tools when selecting crossprotective vaccine strains. Here, we will present antigenic cartography data of H1 and H3 influenza A viruses circulating in pigs, and H3 in horses, in North America and the EU and discuss the antigenic, genetic and disease control implications of our findings.

Acknowledgements

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Surveillance Of Livestock For The Next Pandemic Influenza: Lessons From Influenza In Pigs

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Society should expect that animals farmed to meet the food security needs of the human population globally won’t pose a serious public health threat. However the Nipah virus outbreak in Malaysia with a human case fatality rate in excess of 50% from a zoonotic disease transmitted to people from farmed pigs remains a warning that such can be the case. Molecular analyses of the pandemic H1N1 2009 influenza in people indicate that it too most likely arose in intensively farmed animals. Subsequent studies have confirmed pigs as a reservoir of an increasing range of influenza viruses and, importantly, influenza viral genes that sometimes originated from people and sometimes have been transmitted back to people in newly reassorted viruses. In Australia outbreaks of respiratory disease and deaths in pigs in 2012 have had influenza viruses in the multifactorial aetiology of the porcine disease. Different farms in different parts of the country have been involved, in epidemiologically unrelated incidents. In each outbreak investigation, reassorted influenza viruses with haemagglutinin and neuraminidase genes similar to genes seen in the human population one to two decades previously were detected, inserted on a genetic backbone of the pandemic H1N1 2009 virus. However there has been little public concern in response to these findings, and nationally it is not intended to conduct surveillance to establish the influenza virus status of pigs in Australia. This paper explores the reasons for lack of surveillance in farmed animals internationally and proposes actions that may move to redress the perceived inadequacies.

A reverse genetics approach to vaccine breakdown. Can past outbreaks inform surveillance & vaccine strain selection for equine influenza?


Equine influenza viruses (EIVs) belonging to the H3N8 subtype have caused numerous outbreaks in horses since the first pandemic in 1963. Mandatory vaccination was introduced in the UK in the 1980s but without a mechanism for ensuring that strains were updated. In 1989 there was an extensive outbreak in both vaccinated and unvaccinated animals in the UK, the first example of mass breakdown of vaccination. The haemagglutinin sequences of vaccine strains in use at the time had more than ten amino acid differences within the HA1 sequence when compared with outbreak strains. Site directed mutagenesis and rescue of a panel of viruses by reverse genetics allowed antigenic characterization of individual point mutants and revealed that only one or two changes at specific places were necessary to alter the antigenicity of the HA such that a panel of ferret sera could distinguish between them. Equine sera proved too cross-reactive to distinguish between strains by HI assay, but were applied to virus neutralization assays. Antigenic characterization of point mutants identified specific changes associated with evolution of the European sublineage of EIV, but also identified changes relevant for other sublineages.
Detection Of Airborne Swine Influenza A Under Field Conditions

Information on transmission and airborne detection of influenza A in pigs is scarce, therefore, the objective of this study was to detect influenza A virus from aerosols collected from acutely infected pig populations under field conditions. The study was carried out in two phases. Each phase used two acutely infected pig populations raised under commercial conditions identified through constant communication with local veterinarians. In phase I of the study, detection of airborne influenza A virus happened at the site where pigs were housed whereas in phase II detection was also attempted at specific distances downwind from the site.

In phase I, a total of fifteen 30-minute air samples were collected inside the barn by simultaneously collecting air samples using air cyclonic collectors. For the outside air samples, cyclonic collectors were placed under the pit fan exhaust outlets or besides the ventilation exhaust fans and were allowed to run for a period of 30 minutes. Additionally, 15 oral fluid samples were collected from pigs inside the barn. If a population tested positive in the first visit, a second visit was scheduled seven days after the first visit and testing repeated.

In phase II, the sampling protocol inside the barn was the same as in phase I. As for the outside air samples collected from the exhaust air, thirty 20-minute air samples were collected (15 in the afternoon and 15 in the morning). Additionally, thirty 20-minute air samples were collected downwind at the two closest two public roads approximately 0.5 and 1 mile away from the barn for a total of 60 (30 in the evening – dusk and 30 at dawn – morning) air samples. Air and oral fluid samples were tested for influenza RNA by RRT-PCR. Further diagnostics included virus isolation, titration, subtyping and sequencing.

For phase I, a nursery (Farm 1) was found to be acutely infected with an H1N2 influenza A virus. All air samples (inside and outside) tested positive for influenza A. Virus was isolated from 8 (7 inside and 1 outside) air samples. Virus was also isolated from 11 of 15 oral fluid samples. During the second farm visit, out of 15 air samples collected inside the barn, 6 were classified as suspect and the rest as negative. Out of 15 air samples collected outside, 2 tested positive and 1 was classified as suspect. All 15 oral fluid samples tested positive. No virus was isolated from these samples.

In phase II, a WTF barn (Farm 3) positive for influenza A H1N1 had all but 2 inside air samples positive. As for the outlet air samples, 20 tested positive, 9 were classified as suspect and 1 tested negative. Seven air samples collected 0.5 mile away from the source population were classified as suspect, the remaining samples tested negative. As for the 1 mile air samples, 1 sample tested positive and 2 were suspect. No virus was isolated from any of the air samples. All oral fluids tested positive for influenza. Farm 4, a WTF barn had all the inside air samples positive for influenza HXNX. At the outlets, 26 air samples tested positive and 4 were suspect. Downwind, at 0.5 miles, 6 samples were classified as suspect but at 1 mile downwind 2 samples were positive and 11 were classified as suspect.

Our results show that acutely infected pig populations generate airborne influenza A virus viable particles capable of being exhausted from pig barns and transported downwind. Our results also suggest that influenza aerosol dissemination might be the most likely route for influenza virus regional dissemination. Additionally, acutely infected pig populations can generate viable particles for a number of days. Detection of influenza A virus particles in the field will depend on the course of disease, as it was previously demonstrated. More studies are required to understand the risk of aerosol transmission between farms and the regional dissemination of influenza A virus.

The Genetics Of Virus Particle Shape In Equine Influenza A Virus
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Many human strains of influenza A virus produce highly pleomorphic virus particles that at the extremes can be approximated as spheres of around 100 nm diameter or filaments of similar cross-section but elongated to lengths of many microns. However, the role filamentous virions play in the virus life cycle remains enigmatic. Here, we investigated the budding morphology of equine influenza A viruses. The majority of H3N8 strains were found to produce a mixture of spherical and filamentous virions, as did the prototype H7N7 A/eq/Prague/57 strain. The exception was found to be the prototype H3N8 isolate, A/eq/Miami/63, that has a long history of laboratory reassortment. Reassortment of equine influenza virus M genes from filamentous and non-filamentous strains into the non-filamentous human virus A/PR/8/34 confirmed that segment 7 is a major determinant of particle shape. Sequence analysis of several strains of equine influenza virus identified consistent M1 amino-acid polymorphisms plausibly associated with determining virion morphology and the introduction of these changes into viruses confirmed their importance. However, while the change S85N in the eq/Miami/11/03 and A/eq/Newmarket/1/93 M1 genes was sufficient to almost abolish formation of long bundles of filaments, both N85S and N231D changes were required in the A/eq/Miami/63 gene to restore the phenotype. Thus influenza A viruses from other mammalian species also produce filamentous virions and the genetic determinants are set by the M1 protein. However, the precise sequence polymorphisms
are different to those previously identified in human viruses.

Comparative Pathogenesis Of Influenza A Virus Subtypes; Pandemic H1n1 2009 And Contemporary Swine H3N2, In Pigs And Ferrets.

Background:
Influenza viruses of H1 and H3 subtypes are commonly found in both pigs and humans, albeit evolutionarily removed across the species with the exception of recent introduction of the pandemic(pdm) H1N1 strain. In Europe the H3N2 viruses are of conventional human/avian origin and the H1N1pdm contains genes derived from human/avian/swine/sw of North American and European/Asian origin. The ferret is accepted as a good animal model for human disease, swine also have some utility in this area as well as representing a natural reservoir.

Objective:
To determine correlates of disease pathogenesis in pigs and ferrets for swH3N2 and H1N1pdm.

Methods:
Pigs and ferrets were infected intranasally with 5-6 logs_{10} of either A/swine/Italy/55295/2011 swH3N2 (ISZLER) or A/England/195/09 H1N1pdm (HPA/NIMR) and monitored for the development of clinical symptoms, including pyrexia, virus shedding and respiratory pathology.

Results:
Infection with swH3N2 virus indicated that there were substantial differences in clinical presentation, lung pathology and virus shedding between pigs and ferrets. The latter had reduced replication in the lung, more virus released into the environment from the upper respiratory tract and mild clinical disease. In pigs swH3N2 virus infection did not produce any clinical signs, viral shedding was moderate and lung involvement was substantial. Whereas, H1N1pdm infection presented as more similar across the two species in relation to virus distribution in the respiratory tract, clinical presentation and virus shedding remained enhanced in ferrets compared to pigs.

Conclusions:
The differences in pathogenesis of influenza A in pigs and ferrets were greater for swH3N2 than H1N1pdm. It appears that European H3N2 viruses have reached an equilibrium in their host through viral adaptation over many years but host and viral changes associated with H1N1pdm infection are yet to reach this balance. Both pigs and ferrets represent useful models of disease for each hosts and representing human infection. Ferrets appear to be more sensitive with moderate-severe disease than the mild disease observed in pigs. Ferrets are readily susceptible to a wider range if influenza A subtypes than pigs, the reasons for this continues to be a key area of investigation and is not restricted to virus receptor distribution.

Acknowledgements:

Awareness And Practices Regarding Zoonotic Influenza
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Swine workers play a key role in transmission of zoonotic influenza. At the same time, there are limited prevention programs targeting these workers. This study assesses practices and attitudes regarding zoonotic influenza transmission among swine workers in Romania. Methods A convenience sample was recruited in seven large Romanian swine production facilities. Volunteers completed a survey that included demographics, farm characteristics, tasks performed, and infection control practices. Results 103 workers completed the survey. Workers reported having worked at the farm for 8 years and that their farm housed approximately 9000 pigs. 93% of workers reported awareness of national guidelines for flu prevention and 60% of workers reported having a sick leave policy at work. The percentage of workers reporting concern about contracting influenza for pigs or giving influenza to pigs was 78% and 70% respectively. 5% of workers reported influenza vaccination during 2009-2010, while 7% reported vaccination during the 2010-2011 season. Despite this, only 5% of the workers reported flu like illness during the past year. 3% of workers reported that pigs appeared sick with influenza in the past year. Use of personal protective equipment (PPE) included overalls (91% of workers), rubber boots (84%), and disposable gloves (38%). Workers occasionally used dust masks when cleaning barns, but no workers reported using an N95 or similar respirator. No increased use of PPE was reported when pigs appeared ill. In comparison to a similar survey of US workers, Romanians reported greater concern about influenza but lower rates of influenza vaccine, and use of respiratory protection. Conclusions Despite concern about zoonotic influenza, Romanian swine workers report little use of influenza vaccine or respiratory protection. As part of global pandemic influenza preparedness, enhanced prevention programs for swine workers should address these gaps.
Morphometry For Swine Influenza Pathogenesis Studies
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Increasingly, studies of the pathogenesis of influenza infection and comparisons made using different isolates within the pig model are being used. For these studies, a section of tissues containing the characteristic lobar consolidation is selected (if available) for routine microscopic scoring. However, for more advanced techniques to quantify structural changes and use different staining methods, a more standardized sampling method may be required. Specific aims were to establish standardized sampling sites, score each site independently with set criteria and to compare scores between sites. Sixty-five 4-week-old pigs divided into high (HBW) and low (LBW) birth weight groups were intratracheally inoculated with $10^{6.3}$ TCID50/ml of A/sw/TX/4199-2/1998 H3N2 and euthanized 48h later. Samples were collected 2.5cm from the tip of the left cranial (A), left middle (B), right cranial (C) and right middle (D) lung lobes. The tissue was fixed in 10% formalin for 24h and trimmed so that the histological sample examined came from approximately 2 cm from the tip of the lobe. The sections were scored by a single pathologist using established scoring criteria. ANOVA analysis showed that although there was a trend toward higher scores in the LBW group, there was no significant difference between mean microscopic lesion scores for HBW and LBW groups ($p=0.084$), but there was a difference between lung lobes ($p=0.026$). The post hoc Tukey test showed that mean score for C significantly higher compared to B and D for the 65 pigs. A difference that was more striking in the LBW group alone ($p=0.009$), with C higher compared to the other three lobes. The previously suggested cause for increased titers and pneumonia scores for this lobe has been the fact that the right cranial lung lobe is ventilated separately by the local inflammation leading to impaired immune responses.

The Effect Of Demographic Characteristics On The Humoral Immune Response Elicited By Influenza Vaccination In Donkeys
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Various demographic characteristics may influence the immune response to infection or vaccination with influenza virus. It is increasing recognized that adipose tissue is an active endocrine organ that produces biologically active substances, an excess of which can induce chronic local inflammation leading to impaired immune responses. During the 2009 H1N1 pandemic, obesity was associated with more severe outcome in people. Recent studies suggest that half the UK equine population is overweight. Donkeys are particularly prone to obesity and are reported to succumb to more severe influenza infection than other equidae. The aim of this study was to determine whether markers of obesity were associated with a reduced antibody response to a booster dose of a commercially available inactivated influenza virus vaccine in donkeys. Surplus to diagnostic requirement serum samples from 55 donkeys were assayed for antibodies to A/equine/Newmarket/2/93 by single radial haemolysis. There was no significant association between antibody level and time since vaccination (samples were obtained in a 7–80-day-interval post vaccination). Nor was there any significant association with body condition score, weight, serum levels of triglycerides, adiponectin or tumor necrosis factor-α. However, antibody levels were significantly higher ($p=0.009$) in female than in male donkeys. The implications of this finding and potential mechanisms for this gender dimorphism will be discussed.

Antigenic And Genetic Characterization Of Swine Influenza Virus (Siv) Isolates From Ohio Agricultural Fairs
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The recent emergence of H3N2 variant (H3N2v) influenza A viruses (IAVs) has posed a potential threat to public health by causing swine-origin IAV infections at agricultural fairs across six states in USA. Vaccination is the most effective method to prevent human infections, and understanding antigenic profiles of the SIVs from agricultural fairs will be critical towards developing an effective vaccination strategy. Here 68 H3N2 SIV isolates from seven Ohio fairs (2009 to 2011) and 14 from commercial farms (2006 to 2012) were antigenically characterized. These isolates were compared with other H3 subtypes of avian, canine, human H3N2v, and human seasonal IAVs (1979 to 2005). Antigenic cartography demonstrated that H3N2 SIV isolates from Ohio fairs could be divided into two antigenic groups: the 2009 fair isolates as one, and the 2010/2011 fair isolates with human H3N2v as the other. Two observed antigenic clusters were also shown to be co-circulating in commercial swine populations. All four human H3N2v isolates, all 68 fair isolates, and 13 farm isolates showed different extents of antigenic cross-reactivity with the ferret antisera produced against a few selected human seasonal IAVs from 1982 to 1997 but did not cross react with those against canine and avian IAVs. Genomic analyses demonstrated that the HA genes of these H3N2 SIVs and H3N2v belong to the same genetic lineage (IV), which have been predominant in North American swine population since 2005. Furthermore, the M genes of the 2009/2010 fair isolates were from “classical” SIVs but those of the 2011 fair isolates from the 2009 pandemic H1N1 virus. Our results suggested that H3N2 SIVs continues to evolve both antigenically and genetically after they were introduced from human to swine, and that there were more than one time human H3N2 IAVs spilled over from human to pigs. Our observations also suggest that agricultural fairs can serve as a potential animal-human interface for influenza interspecies transmission; therefore surveillance shall be continued to monitor influenza viruses, including both swine and human influenza viruses, at agricultural fairs.
SESSION IV – EMERGING ISSUES AND NEW DEVELOPMENTS

Salmon Anaemia In Chile
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The infectious salmon anaemia virus (ISAV), belongs to Orthomyxoviridae family with similar characteristics of Influenza A virus. The ISAV was the major cause of the 2008-2009 outbreaks, producing high economical losses in to the salmon industry in Chile. It has been proposed that the virulence of ISAV isolates lies mainly in the hemagglutinin-esterase (HE) and fusion glycoproteins. Phylogenetic analysis revealed that Chilean isolates are related to European ISAVs. The segment 6 encodes the HE protein, which also contains the highly polymorphic region (HPR) sequence in the stalk domain, showing different sequence lengths. Different viral isolates of ISAV have been reported in Chile, the more prevalent are the HPR7b and HPR1c strains. A characteristic of the HPR is the existence of a variant that has all the motifs already described for the other HPRs, of which the largest is called HPR0. It is suggested that deletions in HRP0 may be used as genetic variability by ISAV. Reverse genetic systems are key tool for the study of Influenza virus, however, such system has not yet been described for ISAV. Utilizing a helper virus as complement and a plasmid that allow the expression of segment 6 incorporating a Not I restriction site sequence as marker in the HPR, we have developed a system that has not yet been described for ISAV. Utilizing a helper virus as complement and a plasmid that allow the expression of segment 6 incorporating a Not I restriction site sequence as marker in the HPR, we have developed a system that has not yet been described for ISAV. Utilizing a helper virus as complement and a plasmid that allow the expression of segment 6 incorporating a Not I restriction site sequence as marker in the HPR, we have developed a system that has not yet been described for ISAV. 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showed that C/OK virus displayed a broader cellular tropism than a human influenza C virus. The observed difference in cellular tropism was further supported by structural analysis showing that hemagglutinin esterase (HE) proteins between two viruses have conserved enzymatic but divergent receptor-binding sites. These results suggest that C/OK virus represents a new subtype of influenza C viruses that currently circulates in pigs that has not been recognized previously. The presence of multiple subtypes of co-circulating influenza C viruses raises the possibility of reassortment and antigenic shift as mechanisms of influenza C virus evolution.

Evidence Of Influenza Infection In Synanthropic Non-Human Primates At The Animal-Human Interface

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Several species of macaques (Macaca spp.) are synanthropic, i.e. capable of thriving alongside humans in human-altered environments. These behaviorally and ecologically resourceful nonhuman primates (NHP) can bridge the divide between their natural habitats and areas populated by humans and, because they are so similar to humans genetically and immunologically, they potentially play a critical role in the introduction of novel pathogens, such as avian influenza, from other wildlife reservoirs. Experimentally, NHP are used in the laboratory setting and can be infected with seasonal and avian influenza viruses; however, there is little information on the prevalence of influenza virus infection among NHP in natural settings. In these studies, we investigated the influenza virus seroprevalence in NHP in several parts of the world. We detected both NP-specific and neutralizing antibodies against different seasonal human and avian influenza viruses in sera from macaques sampled in Singapore, Bangladesh, Gibraltar, Cambodia and two sites in Indonesia between 2001 and 2011. Based on these results we then screened respiratory swabs from macaques in Cambodia in 2011 by real-time reverse transcription-polymerase chain reaction and detected M gene in 2.1% of the samples. To date, attempts to isolate an NHP influenza virus have been unsuccessful. Here we provide the first evidence that macaques living in close proximity to humans can be infected with seasonal and avian influenza viruses and our results demonstrate that free-ranging, pet and performing macaques are susceptible to both seasonal and avian influenza virus infections and that both viruses and antibodies are readily detectable in these populations. These data suggest that NHP at the human-NHP interface could represent a “mixing vessel” for the generation of novel strains of influenza virus.

Isolation And Characterization Of A Novel Bovine Influenza C Virus From A Clinical Case

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A bovine respiratory disease (BRD) syndrome case was referred to Animal Disease Diagnostic Laboratories. Multiple bacterial agents were isolated and the presence of several viral pathogens were identified by PCR using a panel of primer pairs. By differential cell propagations and viral specific fluorescent antibody (FA) tests, bovine herpesvirus (BHV) type 3/4 was isolated from the BT cells from sample #3 and parainfluenza 3 (PI3) virus was identified on all three cell types by FA staining from samples # 13 and #15. HRT-18G cells inoculated with pooled #14 sample was negative by all known viral-specific FA staining but positive by indirect fluorescent antibody (IFA) staining using a convalescent bovine serum and anti-bovine IgG FA conjugate. Sample #15, previously tested PI-3 FA positive, was also IFA positive for this unknown virus. Isolate #14 was concentrated and subjected to electron microscopic (EM) examination. The biological and physical characteristics of this unknown virus are similar to a virus in the family paramyxoviridae, however the electron micrograph of negatively stained virus was also consistent with influenza viruses. That the unknown virus is prevalent in cattle was demonstrated when many post-weaned calf serum samples, originating from numerous herds, tested positive by the Isolate #14 IFA test. Viral nucleic acids were isolated from Isolate #14 and sequenced using a NGS sequencing technology. Contigs were assembled from the sequences obtained from NGS after subtraction of known human genomic sequences. By searching the NCBI GenBank database, amino acid sequences of seven gene contigs matched the human influenza C virus. Here we describe the isolation and identification of the first and only influenza C virus of bovine origin that has ever been reported.
Susceptibility Of Swine Influenza Viruses Isolated In The United States During 2009-2011 To Neuraminidase Inhibitors And Adamantanes

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Background: Swine influenza viruses can play an important role in initiating a pandemic through direct human infection or through reassortment and generation of novel variants. Genetic analyses of the human 2009 pandemic H1N1 influenza virus (H1N1pdm09) provided clear evidence of this possibility as the virus was generated by multiple reassortment events between viruses that had circulated in swine for more than 10 years. One option for the control of influenza disease is antiviral treatment. Limited information is available on the susceptibility of swine influenza viruses to specific influenza drugs. Objectives: The aim of this study was to determine the susceptibility of swine influenza viruses circulating in the United States in 2009-2011 to existing antiviral drugs (neuraminidase inhibitors [NAIs] and adamantanes). Methods: The susceptibility of swine influenza viruses to NAIs was assayed in a phenotypic fluorescence-based assay with MUNANA substrate. Molecular markers for NAI and adamantane resistance were determined by sequence analysis of the NA and matrix (M) genes. Results: Active surveillance for influenza virus in swine in the United States in 2009-2011 identified 746 influenza viruses out of 16,170 nasal swabs tested, from which H1N1, H1N2, H3N2, and H1N1pdm09 viruses were detected in 18%, 16%, 7.6%, and 14.5% of the samples, respectively. Phenotypic assays revealed high susceptibility of swine influenza viruses to NAIs, and sequence analysis did not identify NA amino acid substitutions associated with NAI resistance (E119A, H274Y, R292K, and N294S). The incidence of adamantane resistance was caused predominately by a single S31N M2 amino acid substitution and varied among swine viruses of different subtypes: a 100% resistant phenotype was determined for H1N1pdm09 viruses, although only ~20% of H1N2 and ~50% of H3N2 viruses carried drug resistance markers. Conclusions: Natural NAI resistance among swine influenza viruses is rare. The different rates of adamantane resistance among swine influenza viruses suggest evolutionary dynamics of the M gene in that host. The pandemic potential of swine influenza viruses warrants the monitoring of the antiviral susceptibility of these viruses.
Notes
NEGLECTED INFLUENZA MEETING

POSTER PRESENTATIONS
**POSTER 1**

**Establishment Of An Active Surveillance Programme For Eiv In The Uk & Analyses Of Ha And Na Sequences From Multiple Outbreaks**


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Equine influenza viruses are a major cause of respiratory disease in horses worldwide and, like other influenza viruses, undergo antigenic drift. It is therefore important to ensure that strains included in vaccines remain up to date. A sentinel practice scheme of over 190 veterinary practices was established in the UK to encourage the submission of nasal swab samples from horses suspected of having equine influenza. Following positive diagnoses, outbreak alerts were sent out by text message (Merial TextAlert) to participating vets. Tweets were sent from our surveillance Twitter account (@equiflunet) and our surveillance website (www.equiflunet.org.uk) was updated with details about the outbreaks.

Virus isolates from more than 20 outbreaks were characterised antigenically by haemagglutination inhibition (HI) assay against a panel of ferret antisera. HA and NA nucleotide sequences were also determined and compared to vaccine strains and other previous isolates. Genetic analysis of the HA segment illustrated the continuing divergence of the two clades of the Florida sublineage, which was also shown by increasing titre differences during antigenic analysis. In the UK during 2010-2012 only viruses belonging to clade 2 of the Florida sublineage, as classified by their HA segment, were isolated. In 2012 two populations of clade 2 viruses were isolated, one with an amino acid substitution at position 144 of HA1, the other with a substitution at 179. Analysis of NA sequences also showed the divergence of the two clades, in addition to highlighting reassortment events between the two clades. These data demonstrate that the continuing surveillance of equine influenza is crucial in order to maintain epidemiologically relevant vaccine strains.

**POSTER 2**

**Review Of Influenza A Virus In Swine Worldwide: A Call For Increased Surveillance And Research**

OFFLU Swine Influenza Virus Technical Working Group – A. Vincent

Pigs and humans have shared influenza A viruses (IAV) since at least 1918, and many interspecies transmission events have been documented since that time. However, despite this interplay, relatively little is known regarding IAV circulating in swine around the world compared to the avian and human knowledge base. This gap in knowledge impedes our understanding of how viruses adapted to swine or man impacts the ecology and evolution of IAV as a whole and the true impact of swine IAV on human health. The pandemic H1N1 that emerged in 2009 underscored the need for greater surveillance and sharing of data on IAV in swine. In this paper, we review the current state of IAV in swine around the world, highlight the collaboration between international organizations and a network of laboratories engaged in human and animal IAV surveillance and research, and emphasize the need to increase information in high priority regions. The need for global integration and rapid sharing of data and resources to fight IAV in swine and other animal species is apparent, but this effort requires grassroots support from government, practicing veterinarians and the swine industry and, ultimately, requires significant increases in funding and infrastructure.

**POSTER 3**

**Influenza A Virus Hemagglutinin Diversity In Immune Pigs**

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Swine influenza is a type A influenza virus (IAV) recognized for causing respiratory disease in pigs. The mechanisms by which the virus evolves within a host or even within a population are not fully understood (1). There is little information about how the immune pressure, vaccination practices, herd immunity, and viral diversity contribute to the appearance of “new” viruses. The objective of this study was to evaluate genetic changes in the IAV hemagglutinin (HA) of weaned pigs with or without maternal immunity to IAV.

Virus sequences were obtained from two groups of ten three-week-old pigs that were infected upon contact with a weeder pig infected with swine influenza H1N1 virus (A/Sw/IL/0239/04). Pigs in one group were born from sows previously vaccinated with a vaccine created with A/Sw/IL/02450/08 (14% nucleotide difference), and pigs in the other group were born from naïve and unvaccinated sows. Pigs had suckled colostrum from their respective mothers. Nasal swabs were collected daily from each pig and full HA sequences were obtained from positive samples. Synonymous and non-synonymous mutations were assessed and HA1 hypothetical proteins for each sequence were obtained. Predicted HA1 proteins were modeled (2) and each amino acid substitution detected was mapped and compared to the five antigenic sites previously described (3).

Both synonymous and non-synonymous changes were observed in the full HA sequences from immune and non-immune animals. Changes happened in a very short time post infection, in and out of known antigenic sites, within a relative small population, and in pigs of different immune status. Some of the changes observed in this study corresponded to changes previously described in the literature however, it is still not clear what is the biological significance of them under experimental conditions.

**References**


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POSTER 4

Full Genome Of Swine Influenza A Virus In Immune Pigs Using Next Generation Sequencing
Andres Diaz, Anna Romagosa, Shin Enomoto, Marie Culhane, Sirind Sreevatsan, Montserrat Torremorell. College of Veterinary Medicine, University of Minnesota, St. Paul, MN, United States.

Influenza A virus (IAV) is endemic in pigs and worldwide distributed (1). IAV infection-replication fitness may depend not only on the HA and NA characteristics; however little is known about the genetic diversity of the full genome of IAV in pigs. In this study we report the results of sequencing the entire genome of an H1N1 IAV, using the 454 sequencing platform to describe the genetic diversity of IAV across its complete genome during experimental conditions in pigs.

Naïve three-week old pigs were vaccinated against IAV using a commercial vaccine. After vaccination animals were exposed to a seeder pig shedding H1N1 IAV (A/Sw/IA/00239/04). Nasal swabs were obtained from all pigs daily and tested for IAV by RT-PCR (2). Two positive samples from each pig (seeder and contact-exposed) and the virus challenge inoculum were selected to amplify the complete genome of IAV (3) using a high fidelity polymerase. cDNA was purified and submitted for sequencing. A reference sequence for each gene was used to assemble all reads using Newbler Assembler.

Full length sequences were obtained for all 8 segments in all the samples analyzed. For each segment between 169 and 71060 reads were obtained giving enough coverage for each position to assess diversity within and between samples. Results from this study indicate that swine IAV genetic diversity is very dynamic throughout the infection process. Next generation sequencing is proving to be very informative to fully evaluate the degree of virus diversity in animal populations. In addition, the methods used in this study allowed the characterization of swine influenza viruses directly from nasal samples which may help avoid viral selection bias that can occur during virus isolation.

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POSTER 5

Dynamics Of Flu Infection In Sow Farms
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Neonatal pigs play an important role in maintaining swine influenza A virus (IAV) infections in pig populations (1, 2). The objective of this study is to describe the dynamics of virus infection in breeding herds overtime and to evaluate the role of replacement animals and piglets on the introduction and maintenance of IAV in these herds.

Five conveniently selected herds were enrolled November 2011. In each farm 90 nasal swabs were collected in a monthly basis for one year from three subpopulations: suckling piglets, > 30 days gilts on-site and <30 days gilts on-site. Additionally oral fluids were collected from each subpopulation. Samples were tested by RT-PCR for IAV and virus isolation from all PCR positive samples. Phylogenetic analysis was developed to assess the genetic association of flu viruses within and between animal subpopulations found in the same herd.

All farms have tested positive at least once to swine IAV. In all farms piglets have tested positive to IAV at least once. In gilts >30 days on-site, IAV has been detected only in two farms and has only been detected once in each of these farms. In gilts <30 days on-site, IAV has been detected in two farms (once and five times respectively).

Swine IAV transmission in endemic infected herds appears to be very dynamic within and between subpopulations of animals found in breeding herds. Replacement animals can be a source of new viruses for the sow herd or naïve for the resident virus. Newborn piglets are naïve at birth to any IAV and they can become a source of IAV for other pigs at weaning. All of these populations could potentially be mixing vessels for different viruses if a mixed infection takes place resulting in new viruses.

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References
POSTER 6

An elisa for equine influenza - diva or understudy?
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One of the factors that influenced the choice of vaccine in the control and eradication of equine influenza in Australia in 2007 was the ability to use a DIVA to differentiate between naturally infected horses and those vaccinated with a canary pox recombinant expressing only the haemagglutinin. The ID Vet ELISA detects antibodies against the internal nucleoprotein of influenza viruses. This study was undertaken to evaluate the ability of this ELISA to detect an antibody response induced by vaccination with whole inactivated, subunit and canary pox recombinant vaccines against equine influenza, and to detect antibodies elicited by natural infection and by experimental challenge.

Sera collected from 60 weanlings following primary vaccination with five different vaccines (two whole inactivated vaccines, two ISCOM based subunit vaccines and a canary pox recombinant vaccine) were tested by ELISA and Single Radial Haemolysis (SRH). The SRH detects antibodies against haemagglutinin. The ELISA did not detect the antibody response to vaccination with the canary pox recombinant vaccine (Proteq Flu Te), confirming the usefulness of the combination of this kit and vaccine in a “DIVA”. The pattern of antibody response post-vaccination detected with the ELISA was similar to that of detected by the SRH test for the other four vaccines. The antibody response to the other two subunit vaccines (Equip FT and Equilis Freqenza Te) was detected by ELISA, i.e. no DIVA capacity was evident. The ELISA demonstrated similar sensitivity to the SRH in detecting a higher and more durable antibody response in horses vaccinated with the whole inactivated virus vaccine Duvaxyn IE T Plus, than other vaccines.

Paired samples collected from 203 horses on 15 infected premises were tested. Fewer seroconversions were detected by ELISA (25%) than by SRH (43%) or Haemagglutination Inhibition (HI) (41%). The acute samples from the majority of affected horses that were not detected by ELISA were seropositive. The ELISA was capable of detecting seroconversion by seronegative horses earlier than the other assays. This finding in the field was confirmed by the analysis of sera from seronegative ponies in an experimental challenge study. All ponies seroconverted by SRH and ELISA 14 days post challenge but were seronegative by SRH at 7 days post challenge when the majority (85%) had antibodies detectable by ELISA.

POSTER 7

A Distinct Lineage Of Influenza A(H1n1)pdm09 Virus
Anna Germundsson Hauge, Hilde Forberg, Britt Gjers

In September 2009, influenza A(H1N1)pdm09 virus was introduced to Norwegian pigs. The Norwegian pig population was previously naive against this and other influenza A viruses. Sequencing and phylogenetic analyses of viruses circulating in Norwegian pigs during the period of 2009 to early 2011, showed that A(H1N1)pdm09 in pigs closely mirrored the contemporary viruses circulating in humans. In 2011, however, virus persisted in pigs after cessation of human virus circulation. In this study, the evolution of A(H1N1)pdm09 virus in the Norwegian pig population, where no other influenza subtype is circulating, was studied. Nasal swab samples collected from pigs showing clinical signs of influenza infection were submitted to the Norwegian Veterinary Institute and tested for A(H1N1)pdm09 virus by real-time RT-PCR and the HA-gene of positive samples were sequenced. In 2011, positive samples at 5 different time points (mid-April, late-April, July, October and November) were included in the analysis. In addition, human viruses occurring in Norway and obtained by the Norwegian Institute of Public Health, as well as closest matches and a selection of representative international reference strains available in the publicly accessible EpiFlu database provided by the Global Initiative on Sharing All Influenza Data (GISAID), was included for comparison. Sequencing analysis of the HA gene of A(H1N1)pdm09 virus detected in pigs in April and July 2011 only showed minor nucleotide substitution. Moreover, phylogenetic analysis show that the A(H1N1)pdm09 virus circulating in pigs resembled the A(H1N1)pdm09 virus circulating in humans during this time. However, viruses detected in pigs in October and November 2011, were forming a distinct phylogenetic group. This distinct group is characterised by amino acid substitutions N31I, S84I, S164F and N473D in the viral haemagglutinin gene, with the substitutions at positions 164 and 473 appearing to be unique to this group. Amino acid position.

POSTER 8

Role Of C/EBP Homologous Protein-10 (Chop-10) In Influenza A Virus Mediated Inhibition Of IL-23 In The Macrophage Cells
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Secondary bacterial pneumonia is a major complication of influenza A virus (IAV) infection. Recently, using IAV and Staphylococcus aureus co-infection mouse model, inhibition of IL-23 by IAV induced type I interferon has been implicated in enhancing bacterial infection in the lung. An endoplasmic reticulum stress induced transcription factor, C/EBP homologous protein-10 (CHOP-10), has been reported to be crucial in the regulation of expression of IL-23. IAV infection of murine tracheal epithelial cells results in induction of ER stress markers activating transcription factor 6 (ATF6) and ER
Equine Influenza Has Impacted The Equine Industry In South America In 2012

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Equine Influenza (EI) is regarded as the most economically important respiratory disease of horses. The aim of this work is to report the epidemiological picture along with the phylogenetic pattern of the virus detected during EI outbreaks occurred in Uruguay and Argentina in 2012. The first alert was around March 10th, with the occurrence of EI among Thoroughbred horses housed in Maroñas (Montevideo, Uruguay) racing and training facilities. On July 8th, horses showing respiratory clinical signs were detected in La Plata and Palermo racetracks, and on July 10th similar cases were also registered in San Isidro racecourse (all located in Buenos Aires (BA) urban and suburban neighbourhoods). Days after, diseased horses were observed in a breeding farm in San Antonio de Areco (100 km north BA), Azul racetrack (300 km south from Buenos Aires), Tucuman racetrack (1000 km west from BA) and in a Córdoba jumping club (500 Km northwest from BA). On November 23rd, the disease was suspected and lately confirmed in show horses in Esquel, Chubut province, 1000 km south from Buenos Aires. Equine influenza virus infection was confirmed by real time RT-PCR of the matrix (M) gene, in 27 of the 37 nasopharyngeal swabs obtained. Phylogenetic analysis of the haemagglutinin (HA) gene revealed that EI virus circulating in Uruguay and Argentina is closely related to Clade 1 of the Florida sublineage within the American lineage, identical to those identified in Kentucky and New...
York in 2011. The origin of the virus has not been precisely identified; nevertheless, in mid-March the Brazilian press reported that 50% of the horses had been withdrawn from races in Porto Alegre and Curitiba (Brazil) racetracks due to an acute respiratory disease. Horses from these regions had been moved to Uruguay, and from Uruguay to Argentina for racing purposes. The negative economic consequences of this reintroduction of EI virus resulted from the withdrawal of several horses from races, from temporary cancellation of race meetings and from the ban on the movement of horses both at national and international level.

POSTER 11

Testing For Equine Influenza Virus Shedding As A Requisite During The Pre-Export Quarantine

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Equine Influenza (EI) is regarded as the most economically important respiratory disease of horses. In the past, countries as Hong Kong (in 1992), South Africa (in 2003), Australia (in 2007) and recently Uruguay and Argentina, have experienced epizootics of EI associated with the import of sub-clinically infected horses, which usually shed only small quantities of virus. During the 2007 outbreak in Australia, real time RT-PCR (qRT-PCR) was used in the mass screening of horses, not only to confirm diagnosis but also to demonstrate that EI virus had been eliminated from the Australian horse population. In order to reduce the risk of virus entry, some countries (United Arab Emirates and Uruguay) have been requesting individual proof of freedom of EI infection before importing horses from Argentina. The aim of this study was to determine the performance of a pan-reactive influenza type A qRT-PCR targeting the matrix gene to certify freedom of EI in healthy horses during the pre-export quarantine. The detection of EI virus RNA was performed on nasopharyngeal swabs obtained both from horses showing respiratory clinical signs and pyrexia during the 2012 EI outbreak in Uruguay and Argentina (n=40), and from healthy horses, not epidemiologically related, during pre-export quarantine (n=103). In order to determine the sensibility of the assay and to generate a standard curve, log dilution of an A/eq/ Argentina/12 influenza virus containing 10^8.00 embryonated hens’ eggs infective doses 50%/ml was included as reference. A sample was considered negative if the Ct value was higher than 38, inconclusive if the Ct was between 36 and 38, and positive if the Ct was under 36. All the samples were run in duplicates. Twenty eight (70 %) of the samples obtained from horses showing respiratory clinical signs gave Ct values between 18.75 and 34.31 (positive). Ninety four (91%) of the samples obtained from horses at the pre-export quarantine gave Ct values higher than 38 (negative), and 9 (9%) gave Ct values between 36 and 38 in one of the duplicates (inconclusive). Thus, this pan-reactive influenza type A qRT-PCR assay performed on nasopharyngeal swabs proves to be a very accurate and suitable test to ensure that clinically healthy horses are not shedding EIV at the time of export.

POSTER 12

Epidemiological Survey Of Swine Influenza A Virus In Wild Boar Population In Two Italian Provinces

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The Wild Boar is recorded in 95 of the 107 Italian provinces, according to a very approximate estimate, based on annual number of animals stalked, there are no less than 600,000 wild boars throughout Italy. In many provinces this species is both hunted and culled. The aim of this study was to provide a preliminary overview on the epidemiological data of swine influenza virus (SIV) infection in wild boar population in contiguous pre-Apennic areas of Parma and Piacenza provinces in Northern Italy, where wild boar is wide spread and with large population. Since July 2012 to December 2012, during hunting and culling programs, 300 lungs samples were collected from wild boar stalked in Parma (n=193) and Piacenza (n=107). The samples were submitted to Real Time PCR for gene M of SIV. The samples resulted positive to PCR tests were submitted to virological tests by inoculation on embryonated hen’s eggs and on cell lines. SIV isolates were subtyped by Multiplex RT-PCR and submitted to antigenic characterization against reference antisera in inhibition of haemagglutination tests. Full genome of the isolate 291320/2012 was amplified and sequenced. The real time PCR tests on 300 lung samples detected 5 SIV positive samples (1.66%). Molecular biological investigation of the haemagglutinin and neuroaminidase identified the isolates as avian-like SIV subtype H1N1. Antigenic characterization tests confirmed this result. Phylogenetic analysis of the sequences obtained from isolate 291320/2012 showed it clustered with recent Italian avian-like H1N1 SIV isolated from domestic pigs. This study suggests that SIV actively circulates in wild boar population in the investigated area, even if the number of observations is not so high respect to the number of wild boars present and stalked in the
Effect Of Feed Restriction On Outcomes Of Swine Influenza Virus Infection In Pigs Pre-Infected With Mycoplasma Hyopneumoniae

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European avian-like swine H1N1 was identified as one major pathogen of the porcine respiratory disease complex (PRDC) together with Mycoplasma hyopneumoniae (Mhp), and experimental pre-infection of pigs with Mhp was shown to increase the severity of a subsequent infection with H1N1. In order to evaluate novel control strategies towards such a multifactorial disease, we studied the impact of a moderate feed restriction on the ability of Mhp/H1N1 co-infected animals to resist the infection, as efficiency of inflammatory responses might be influenced by the nutritional status of the animal. Two groups of 8 SPF pigs were intra-tracheally inoculated with Mhp and H1N1 21 days apart. One group was fed ad libitum whereas the other one was applied a 40% feed restriction one week before H1N1 infection. Two similar mock-inoculated groups of 4 pigs each were included. All pigs were fitted with a jugular catheter. Three days post-H1N1 infection, the same amount of a standardized meal was given to all animals and kinetics of blood samples were performed during 4 hours for measuring plasma postprandial nutrient concentrations. Pigs were slaughtered 7 days post-H1N1 infection. Clinical signs were observed throughout the study and pathogens were detected in nasal swabs and lung tissues. Feed restriction had no effect on pathogen excretion and dissemination in infected host or on pulmonary lesions. However, feed-restricted pigs presented a shorter hyperthermia as well as a positive mean weight gain over the 3 first days following H1N1 infection as compared to animals fed ad libitum which lost weight during that period. Both infection and feed restriction modified postprandial kinetics of glucose and amino acid concentrations showing dramatic changes in nutrient metabolism. Our results indicated that feeding practices could be a strategy to prepare animals to overcome an influenza infection, especially in a PRDC context.
POSTER 15
Unraveling Exposure – Simultaneous Screening For Antibodies Against Different Influenza Virus Subtypes Using A Protein Microarray
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Objectives
Influenza is a zoonotic disease potentially causing high mortality in poultry and high morbidity in swine. Human cases resulting from spillover of animal influenza viruses are regularly reported. The recent emergence of swine A(H3N2) variant virus and occasional infections with different avian influenza subtypes of varying severity have resulted in many scientific investigations. Population studies examining past exposures to influenza viruses are mainly focused on the detection of serological evidence in either humans or animals, whereas studies bridging human- and animal populations are scarce. To facilitate serological population screening of humans as well as animals, we developed a multiplex screening technique for different influenza subtypes that is operational for the use in humans. For future studies targeting the human-animal interface current work focuses on the adaptation of this technique for the use in chickens and swine.

Methods
We developed a microarray comprising of 22 recombinant HA1-proteins (Immune Technology Corp, USA) representing 13 different subtypes. Antigens were spotted onto 16-pad nitrocellulose slides (Oncyte Avid, Grace Biolabs) using a Piezorray non-contact spotter (Perkin Elmer, MA, USA). Following spotting, the slides were dried overnight and serum samples were subsequently analyzed according to a standardized protocol.

Results
The microarray was validated using sera from specific pathogen free chickens experimentally infected with different influenza strains. The field samples under investigation in this study were collected in course of outbreak investigations or routine serosurveillance. Subtypes H6 (two outbreaks), LP H7N3 (one outbreak) and H9N2 (one outbreak) were screened as well as a panel of negative sera from a 6-week-old field broiler flock. All sera were confirmed sero-positive/-negative by HI-assay. The protein microarray reliably discriminated between different subtypes and negative samples, thereby showing a high sensitivity and specificity. Likewise, first experiments for the use in swine against known swine influenza viruses look promising.

Conclusions
Our results show that the protein-microarray technique can enhance regular influenza screening in chicken flocks by providing subtype-specific information about past influenza infections while requiring less amounts of sera and time than conventional methods. The microarray technique thereby allows broadening the scope of serological investigation beyond subtypes already known to occur in an animal species. Furthermore, the technique can potentially be adapted for the use in other animal species if suitable species-specific conjugates are available. Work to adapt the microarray for the use in swine is currently ongoing.

POSTER 16
Genetic Signatures Of Virulence In The Influenza A Pb1-F2 Proteins From Non-Avian Animal Reservoirs
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Background: Influenza A virus PB1-F2 protein was shown to contribute to the pathogenesis of both primary viral and secondary bacterial infections. Amino acids (a.a.) L62, R75, R79, and L82 of Pb1-F2 were found to promote virus pathogenicity. These specific a.a. may serve as genetic signatures to predict viral severity. Prior to the emergence of influenza viruses in humans, animals were the natural reservoir. The L62, R75, R79, and L82 a.a. are found in the majority of Pb1-F2 proteins from highly pathogenic avian influenza A viruses of the H5N1 subtype. The role of other (than birds) animal hosts as a source of virulent Pb1-F2 variants has not yet been determined.

Objective of the study: Evaluate the non-avian animal hosts as a reservoir of Pb1-F2 proteins with genetic signatures (such as a.a. L62, R75, R79, and L82) of virulence.

Methods: The predicted a.a. sequences of Pb1-F2 proteins from a total of 658 swine (13 are H5N1), 94 equine, and 30 canine influenza viruses of all lineages available in the Influenza Research Database were examined for the presence of L62, R75, R79, and L82 a.a.

Results: All four virulent a.a. were found in the Pb1-F2 proteins in 3.2% of swine (all of the H5N1 and 8 of the H3N2), one equine (H9N2) and 40% canine (H3N2, H3N8, and H9N2) influenza viruses. Combinations of any three virulence markers were determined in the Pb1-F2 proteins in 3.5% of swine (H3N2 and H3N8), 98.9% equine (H3N8 and H7N7), and 16.7% canine (H3N8) influenza viruses. The vast majority of Pb1-F2 proteins had the L62, R75, and R79 combination. Approximately 26% swine (H3N2) and 43.3% canine (H3N8) influenza viruses had Pb1-F2 proteins with two virulent a.a. The combination of L62 and L82 was typical for swine, while the combination of L62 and R75 was most common for the canine host. The presence of one Pb1-F2 virulence marker (predominantly L82) was observed in 53.6% swine influenza viruses (H1N1).

Conclusion: The non-avian animal hosts possess virulent Pb1-F2 variants suggesting a need for surveillance of influenza viruses with genetic signatures of virulence which may be emerging from these reservoirs.
Zanamivir Conjugates For Influenza Detection
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Objective: The emergence of oseltamivir resistance has caused serious concern of public health. A quick test to detect oseltamivir resistance will help doctors to prescribe proper drugs for influenza treatment.

Methods: We synthesize a zanamivir–biotin conjugate containing a reporting unit of biotin. Both zanamivir and oseltamivir target the neuraminidase on the surface of influenza virus. Oseltamivir sensitive virus can compete out the zanamivir–biotin conjugate to give signal on subsequent treatment with alkaline phosphatase-linked streptavidin. Our drug Resistance Assay by Binding Competition (RABC) can be modified to a quick test on membrane for visual detection.

Results: We used RABC method with zanamivir–biotin conjugate to examine the seasonal influenza clinic samples of years 2005–2009 in Taiwan and 2009–pandemic viruses. The results indicated no oseltamivir resistance occurred until late 2008. However, most seasonal influenza viruses in 2009 and ~40% of 2009–pandemic viruses were oseltamivir resistant strains, for example, H275Y mutants. The arbitrary PCR tests of selected samples also confirmed our results.

Conclusions: We have devised an efficient method using zanamivir–biotin to evaluate the oseltamivir susceptibility of influenza viruses. This is a general method without using specific antibody in immunoassay even for unknown resistant strains.

Pathogenesis And Transmission Of Influenza In Pigs (Flupig)
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Pandemic influenza viruses come from wild birds, but must adapt to efficient replication and transmission in humans to cause a pandemic. Pigs are considered intermediate hosts in which avian viruses adapt to mammals before transmitting to humans. However, their exact role is unclear, as is the nature of the genetic changes that are required for (a) efficient replication of an avian virus in pigs, (b) efficient transmission of avian viruses between pigs and (c) virus transmission from pigs to humans and between humans. FLUPIG, a Framework Program (FP)7 project supported by the European Commission (GA-no. 258084), aims to examine the role of both the role of adaptive mutations and genetic reassortment between viruses in each of these three phases. In addition, the role of host and environmental factors in adaptation is being studied. The occurrence and severity of a pandemic also depends on the immune status of the human population. FLUPIG studies the extent of cross-protection between antigenically different influenza viruses of the H1N1 subtype (heterovariant cross-protection), and between influenza viruses belonging to different haemagglutinin subtypes (heterosubtypic cross-protection). Also, the immune mechanisms required for a broad cross-protection are studied. In addition, we will evaluate the capacity of novel generation vaccines to broaden cross-protection. Most studies are performed in pigs, in other relevant animals, or in explants of the porcine and human respiratory tract, which show maximal similarity to the in vivo situation. Our studies will enable us to advice public health authorities about the role and risk of the pig in the emergence of novel influenza viruses in humans. Combined with improved surveillance for influenza in animals, effective vaccines and antivirals, this knowledge will be critical to control future influenza pandemics.
Asia, the outbreak of H3N8 resulted in significant on-line information dissemination. In general, reports of scientific findings in the popular media most often accurately reflect the details of the findings. This popular media reporting, however, can include editorial commentary that confuses the public, or provides invalid interpretation of scientific findings or recommendations. The author used Google.com to identify 157 web pages related to the H3N8 outbreak in dogs that were posted between September of 2004 and December of 2005, that used the term ‘canine influenza’ in the page title or snippet, and that targeted the general public. Links to articles in scientific journals were not included in the analysis. The highest level of interest in the topic, based on Google Trends query share statistics, was between mid-September and mid-November of 2005, a full year after the outbreak was identified. Authoritative sources were accessible during the period studied, including 1 media briefing posted by the CDC, 11 media posts by 11 universities, 9 state and local governments, and 57 veterinary clinics. The results also included content provided by non-official sources, primarily breed interest groups, traditional news outlets, and humane organizations. This content was at times inconsistent with scientific findings and recommendations, including inappropriately linkage of the H3N8 outbreak to the H5N1 outbreak, unsubstantiated comments regarding transmission, and suggestions that official reports were untrue regarding the danger to humans. Veterinarians and health officials should stay abreast of information disseminated to the public, particularly easily accessible and unofficial on-line content, in order to dispel inaccurate information and prevent inappropriate or unnecessary actions by pet owners to protect themselves and their pets.

POSTER 20
What To Do With Flu At The Fair
Jon Ertl, Marie Culhane.

During the 2012 Minnesota State Fair (MSF), concerns regarding increased risk for influenza interspecies transmission among those attending agricultural expositions became national headlines when the Centers for Disease Control and Prevention reported numerous cases of H3N2v influenza A virus (IAV) in people with exposure to pigs at other state and county fairs. The CHS Miracle of Birth Center (MOBC) at the MSF is filled with a variety of adult and newborn animals including a swine exhibit that consists of 12 pregnant gilts from a single Minnesota farm. The MOBC sponsors and volunteers were vaccinated at 4-5 and 2-3 weeks pre-farrow with IAV vaccine (HO), heterologous IAV vaccine (HE), and no IAV vaccine (controls). Experimentally infected pigs were vaccinated at 4-5 and 2-3 weeks pre-farrow with IAV vaccine, HO vaccine was created using the H1N1 challenge virus for this study and HE vaccine was created using a distinct (<87% HA gene similarity) H1N1 virus. Offspring of vaccinated sows were confirmed to be seropositive to IAV and purchased for use in this study. For each of two or three replicates, 10 pigs (3-4 weeks old) from each of the aforementioned groups were challenged with IAV via direct contact with an experimentally infected pig that was introduced into each group. Experimentally infected pigs were infected with virus HO. Nasal swabs were collected daily for two weeks following direct contact challenge and tested for IAV via RRT-PCR. The transmission parameters (β), infectious period, and reproduction ratios (R) were estimated and compared between groups. Clinical signs, macroscopic and microscopic lesions, and antibody titers were assessed.

Results
IAV testing was negative on throat swabs of 11 MOBC volunteers who had close contact with sows and newborn pigs for extended periods of time. There were no ill MOBC swine. However, in the nearby swine barn, ten pigs had respiratory disease. Of those, six were IAV PCR negative, H1N2v IAV was isolated from three, and H3N2v isolated from one. Also, human infections did occur in people that visited the swine barn, specifically four H1N2v and two H3N2v infections. There were no human illnesses associated with the MOBC.

Discussion
IAV negative MOBC results suggest the precautionary measures may have decreased IAV interspecies transmission at the MOBC or that the small MOBC group of adult females and newborn piglets were less likely to shed IAV. During the 12 days of the MSF, in the swine barn, located 2 blocks west of the MOBC, 4 pigs were IAV positive. Subsequently six individuals who spent prolonged time in the swine barn became sick and tested positive for IAV that were identical to the pig strains. Better ways to manage and minimize IAV are needed to insure the safety of the public and the pigs and to maintain the important goals of county and state fairs, that is, engaging and educating the public about agriculture.

POSTER 21
The Impact Of Maternally Derived Immunity (MDI) On Influenza Virus Transmission In Neonatal Pig Populations
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Introduction: Vaccination of sows and gilts is commonly practiced to control influenza A virus (IAV) in large U.S. farms. Such vaccinations should not only provide active immunity to the breeding herd, but also passive immunity through maternal antibody transfer via colostrum to the progeny. The objective of this study was to assess the role of MDI in reducing IAV transmission in neonatal pig populations.

Materials and methods: IAV negative breeding sows were assigned to one of three treatment groups: homologous IAV vaccine (HO), heterologous IAV vaccine (HE), and no vaccine (NV). Sows within the respective treatment groups were vaccinated at 4-5 and 2-3 weeks pre-farrow with experimental killed IAV vaccines. HO vaccine was created using the H1N1 challenge virus for this study and HE vaccine was created using a distinct (<87% HA gene nucleotide similarity) H1N1 virus. Offspring of vaccinated sows were confirmed to be seropositive to IAV and purchased for use in this study. For each of two or three replicates, 10 pigs (3-4 weeks old) from each of the aforementioned groups were challenged with IAV via direct contact with an experimentally infected pig that was introduced into each group. Experimentally infected pigs were infected with virus HO. Nasal swabs were collected daily for two weeks following direct contact challenge and tested for IAV via RRT-PCR. The transmission parameters (β), infectious period, and reproduction ratios (R) were estimated and compared between groups. Clinical signs, macroscopic and microscopic lesions, and antibody titers were assessed.
Results: All pigs in groups HE and HO had detectable antibody titers to the respective vaccine antigens prior to direct contact challenge. Pigs in group NV were seronegative prior to direct contact challenge. All contact pigs became infected in groups HE and NV and the R estimates did not differ significantly at 7.8 (4.6-12.6) and 11.0 (6.9-16.8), respectively. One pig in group HO became infected following contact and the R estimate was significantly lower in group HO at 0.84 (0.05-3.7) versus groups HE and NV.

Discussion: This study indicates that vaccine induced MDI may be able to reduce IAV transmission in population settings; however, the impact will depend on the priming antigen. This study also indicates that IAV will spread rapidly in non-immune pig populations.

POSTER 22
Validation Of A Commercial Blocking Elisa For Detection Of Influenza A Nucleoprotein Antibodies In Canine Sera
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Canine populations are susceptible to infection by influenza A viruses originating from horses, avian species, and humans. Concerns about the impact of influenza infections on canine health and the role of dogs in the global ecology of influenza A viruses highlight the importance of surveillance in dogs. Serological diagnosis of infection is typically performed using viral subtype specific-assays such as hemagglutination inhibition and virus neutralization, both of which are technically challenging and unsuitable for large-scale surveillance.

Surveillance studies in other species depend on detection of antibodies to the highly conserved influenza A nucleoprotein (NP); however, no such NP antibody assay is approved for canine use in the U.S.A. The purpose of this study was to determine the diagnostic accuracy of a commercial blocking ELISA used for avian species in detecting influenza A NP antibody in dogs. Since the blocking ELISA is not a species-specific or viral subtype-specific format, we hypothesized that it would detect NP antibodies in dogs infected by influenza A virus.

Serum samples from uninfected dogs (n=204) and dogs naturally infected with canine influenza H3N8 virus (n=150) were tested using the IDEXX FlockChek blocking ELISA for influenza A NP antibody in poultry according to manufacturer instructions. The sample/negative control (S/N) absorbance ratios for infected dogs ranged from 0.12 to 0.67 compared to 0.53 to 1.40 for uninfected dogs. A receiver operating characteristic (ROC) curve analysis determined optimum diagnostic sensitivity (99.3%) and specificity (99.0%) at an S/N cutoff ratio of 0.647. Using this cutoff ratio, the overall diagnostic accuracy was 99.2%. Coefficients of variation for intra-assay (4.7%) and inter-assay (6.1%) testing demonstrated good repeatability with canine sera.

The excellent diagnostic accuracy of the commercial blocking ELISA makes it a suitable screening tool for large-scale surveillance for influenza A virus infections in dogs.

POSTER 23
Transversal Study In 9 Swine Farms In Argentina
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Influenza A viruses are important pathogens responsible for economic losses to swine industry and represent a constant threat to public health. In Argentina, the disease was first detected in a swine farm in 2008. Since then, three different subtypes were characterized: pdmH1N1, H1N2 cluster and human origin H3N2. In this work we evaluate by clinical, serological, virological and pathological studies the prevalence and dynamics of IAV infection from commercial swine farms in Argentina. Nine farms with a total of 21,180 sows, representing 10% of the local stock, were included. Nasal swabs and blood samples were obtained every 3 weeks in each farm. For serological studies an ELISA test anti-NP and HI assay were used. Virological studies included viral RNA detection by real-time RT-PCR. Positive samples were processed for virus isolation in MDCK cells and isolated viruses were characterized. Clinical signs compatible with influenza infection were observed in 8 farms. All farms were positive for IAV. Intra-farm seroprevalence was of 48.45%. Sows and fatteners of 160 days old showed the highest mean percentage of positive pigs; however the range of positive animals varied among farms. Also there was not always a direct relationship between percentage of seropositive pigs, clinical signs and virological detection from nasal swabs. Maternal antibodies dropped down between 21 and 35 days old and then seroconverted between 63 and 70 days old. Influenza virus was detected from nasal swabs in 7/9 farms. In 5/7 positive farms IAV was isolated. In addition, 4/8 lungs samples presented pneumonic lesions and IAV was isolated from those lungs. The molecular analyses revealed that pdmH1N1 subtype was present in 4 farms, and a reassortant between H3N2 cluster 2 and pdmH1N1 was present in one farm. The HI results showed that this farms presented antibodies against pdmH1, H3 and H1 cluster . This study contributes with information about the epidemiology of IAV in Argentina.

POSTER 24
Recent Advances In Equine Influenza Vaccination: A Systematic Review
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Equine influenza (EI) is a major respiratory disease of horses, which is still causing substantial outbreaks worldwide despite several decades of surveillance and prevention. Alongside quarantine procedures, vaccination is widely used to prevent or limit spread of the disease.

Objective: This systematic report reviews the different experimental and commercial EI vaccine technologies, their mode of action and the advances achieved during the last few years. Some of the mechanisms behind the
inefficient or sub-optimal response of horses to vaccination will also be discussed.

**Method:** This systematic review regroups articles published in peer-review journals about EI vaccination. Publication date ranged from 2006 to the present time. The PRISMA guidelines were consulted for the preparation of this review.

**Conclusion:** The panel of EI vaccines commercially available is probably one of the most varied, including whole inactivated virus vaccines, Immuno-Stimulating Complex adjuvanted vaccines (ISCOM and ISCOM-Matrix), a live attenuated equine influenza virus (EIV) vaccine and a poxvirus-vectored vaccine. Several other strategies of vaccination are also currently being evaluated. Results from cross-protection studies also indicate that the majority of EI vaccines commercially available would provide protection if use in the face of an imminent outbreak, when boost immunisation and overall increase of herd immunity is essential. However, current duration of protective immunity induced by non-updated EI vaccines is questionable. The frequency of EI outbreaks in recent years, the continuous antigenic variation of EIV and the occasional cases of vaccine breakdown clearly indicate that a strong EI surveillance has to be maintained. The necessity to update the EIV strains currently contained in commercialised vaccines is increasingly pressing. A better understanding of the factors that influence vaccination efficiency and a targeted monitoring of the antibody response prior to immunisation, as recently suggested, would benefit overall protection against EI.

**POSTER 25**

**Surveillance Study Of Swine Influenza Virus (Siv) 2009 – 2012, Pandemic Influenza Virus**

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The pandemic A/H1N1 influenza viruses emerged in both Mexico and the United States in March 2009, were transmitted efficiently in the human population, and spread quickly throughout the world. In the United States, the H1N1 classical swine influenza, designated as H1α, was first characterized in 1931 and dominated through 1998 when H3N2 triple reassortant viruses appeared in swine. Between 1999-2005 three other lineages of H1, H1β, H15, and H1γ, were described. In this study we report evolutionary changes in the hemagglutinin (HA), neuraminidase (NA), matrix (M), and nonstructural (NS) genes from swine influenza viruses from selected cases submitted to the Animal Disease Diagnostic Laboratory in Indiana. The viral RNA genome was converted to cDNA and subsequently RT-PCR and sequence analysis was performed. Surveillance data demonstrated the first appearance of the H1N1 2009 pandemic genotype during 2010. Moreover, the phylogenetic analysis on viruses isolated during 2009 demonstrated 66.6 % of the viruses were subtype H15 with an M gene of swine origin. During 2011, 50% of the viruses grouped with subtype H3 cluster IV in a phylogenetic tree. There was also an increased appearance of the H1N1 2009 pandemic M gene in both H1γ and H15 serotypes. The analysis of the M gene in cases tested during 2012 demonstrated 90% belong to H1N1 2009 pandemic regardless of HA subtype.

Our data indicate that from the samples tested at ADDL, the first detection of H1N1 2009 pandemic genotype in Indiana was during 2010. However, the M gene from the H1N1 2009 pandemic virus, which has been circulating in 98% of influenza A/H1N1 strains in North America, appeared in H3N2 reassortant viruses in Indiana during 2012 in swine populations. Moreover, our data revealed that the predominant HA subtype in samples tested during 2009 were dominated by H16 serotype and suggest that during this period of time the transmission of the H1N1 2009 pandemic strain influenza virus happened from human to swine populations, perhaps contributing to the introduction of the pandemic strain in swine.

**OUTBREAK 26**

**Outbreak Of Influenza A (H3n2) At A County Fair**

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**Introduction**

During the summer of 2012 swine influenza A outbreaks and viral transmission between species occurred at fairs. Samples collected from pigs and people in attendance at the fairs were tested for the presence of influenza A virus.

**Materials and Methods**

Nasal and oropharyngeal samples were randomly collected from pigs. Samples were sent to the State Animal Disease Diagnostic Laboratory where they were tested for the presence of influenza A and the nucleic acid subtype. Samples were forwarded to the National Veterinary Services Laboratories (NVSL) for rRT-PCR to detect the matrix gene from the 2009 pandemic H1N1 influenza virus. Viral genomes were sequenced from nasal swab material using the Ion Torrent Personal Genome Machine. Diagnostic specimens were collected from people reporting influenza-like illness that had been in contact with pigs at the fair and sent to the State Department of Health Laboratory for preliminary testing. Samples were forwarded to the Centers for Disease Control and Prevention (CDC) for further analysis.

**Results and discussion**

Both swine and human specimens were positive for
influenza H3N2 and contained the pandemic matrix gene. Comparison of the sequence obtained by NVSL and CDC indicated virtually identical sequence, demonstrating virus transmission from pigs to people. Influenza H3N2 viruses with the pandemic matrix gene have been found in pigs in multiple states based on swine influenza surveillance testing by State veterinary diagnostic laboratories. Swine influenza is endemic in pig populations throughout the world. Transmission of influenza virus has been documented to occur between pigs and people, particularly when they come in close contact. This report demonstrates the importance of influenza surveillance and the need for strong ties between public health and animal agriculture at the local, State, and Federal levels to rapidly investigate and control outbreak situations.

POSTER 27

National Surveillance For Swine Influenza Virus In The United States, 2009- Present
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Introduction. In April 2009, a National surveillance plan for swine influenza virus in swine was implemented in the United States. Initial focus of the surveillance was to detect the presence and distribution of viruses (especially the 2009 H1N1 pandemic influenza, A(H1N1)pdm09) that are or may be of public health concern. The current objectives of the National Surveillance Program are: [1] Monitor genetic evolution of endemic swine influenza viruses to better understand endemic and emerging influenza virus ecology; [2] Make virus isolates available for research and establish an objective database for genetic analysis of these isolates and related information; and [3] Select proper isolates for the development of relevant diagnostic reagents, updating diagnostic assays, and vaccine seed stock products.

Materials and Methods. There are three components of influenza surveillance in swine; [1] Surveillance of swine with influenza-like illness (ILI) on farms from which samples are submitted for laboratory testing; [2] Surveillance of swine epidemiologically linked to a human case of novel influenza virus, and [3] Surveillance of swine observed with signs of ILI at first points of concentration or comingling events, particularly where there is potential high exposure to humans. Surveillance data include total animals and specimens tested, state of origin of the samples, reason for submission, tests conducted on the samples and their results, as well as sequence information if virus was isolated.

Conclusions and Discussion. Data analysis is underway at the time of this abstract submission. An up to date summary of the surveillance data collected through the United States National Swine Influenza Surveillance Program will be presented at the meeting.

POSTER 28

Isolation Of Influenza A (H3n2) From Farmed Mink
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In summer 2012, two separate mink farms, approximately 100 miles apart, were positive for influenza A (H3N2) virus. Affected mink showed respiratory illness with the major clinical sign of intermittent coughing. In a population of 77,000 mink on the initial farm, at least 25% showed clinical signs with 4% mortality for those affected. There was no human illness associated with the mink farms. Dead affected animals were sent to the Wisconsin Veterinary Diagnostic Laboratory for necropsy. Additionally, lung tissue and pharyngeal swabs were taken on farm. Positive samples were forwarded to the National Veterinary Services Laboratories (NVSL) for virus isolation and sub-typing. Virus isolates were sequenced using the Ion Torrent Personal Genome Machine.

Gross and histopathological analysis indicated rhinitis and severe acute bronchointerstitial pneumonia. Real-time RT-PCR and virus isolation identified influenza A strains with the 2009 pandemic matrix gene and H3N2 hemagglutinin/neuraminidase subtype. Further characterization by sequence analysis of the isolates indicated two distinct H3 viruses, one of which was >99% similar to the influenza A (H3N2) virus causing illness in pigs and humans at fair events in the Midwest United States. The nucleotide sequence of the hemagglutinin gene from the second mink strain was approximately 95% similar with the first strain.

Both farms obtained pork by-products for feed, including lung, from the same packing plant. This centrally located plant draws pigs for slaughter from many Midwestern states and thus could have more than 1 distinct strain in the animal by-products. Use of uncooked offal is a likely source of infection and demonstrates the dangers of feeding animal by-products in the transmission of influenza virus between species.
Antibodies against haemagglutinin as measured by single radial haemolysis (SRH) correlate with protection against equine influenza. However factors other than humoral antibody responses play a role in providing protection against the disease. Previous studies using interferon gamma (IFN-γ) as a marker for a cell mediated immune response showed an increase in IFN-γ synthesis following vaccination with ISCOM based and canarypox recombinant vaccines. The object of this study was to compare the humoral and cytokine responses following vaccination with an ISCOM based vaccine (Equilis Prequenza TE), a canarypox recombinant vaccine (ProteqFlu-Te) and a conventional inactivated whole virus vaccine (Duvaxyn IE-T Plus). Forty four seronegative Thoroughbred weanlings were vaccinated (V1 and V2) 29 days apart. Antibody response was monitored for three weeks post V2 by SRH. The pattern of antibody response was similar for all vaccines i.e. the weanlings responded poorly to V1 but mounted a superior response to V2. The antibody response of the horses vaccinated with the inactivated whole virus vaccine was significantly higher than that of the horses vaccinated with the ISCOM based and canarypox recombinant vaccines. The antibody responses of weanlings vaccinated with the ISCOM based and canarypox recombinant vaccines were similar. In this study 39% of weanlings failed to seroconvert post V1. The poor responders were observed in the weanling groups vaccinated with the ISCOM based and canarypox recombinant vaccines but not in weanlings vaccinated with the inactivated whole virus vaccine. PAXgene blood samples were collected on days 0, 2, 7 and 14 following V1. Gene expression levels of IFN-γ, the pro-inflammatory cytokine IL-1β and a B cell stimulating cytokine (IL-4) were measured using RT-PCR. Mean gene expression levels of IL-1β and IL-4 peaked on day 14 post vaccination. IL-1β gene expression in horses vaccinated with the inactivated whole virus vaccine was significantly higher than in those vaccinated with the other two products. Vaccination with all three vaccines resulted in an increase in IFN-γ gene expression which peaked at 7 days post vaccination. There was no significant difference in IFN-γ gene expression between the whole inactivated, the subunit and the canary-pox recombinant vaccines included in this study.
**POSTER 31**

**The Use Of Equine Influenza Pseudotypes For Serological Screening**

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Standard assays used for influenza serology present certain practical issues, such as inter-laboratory variability, complex protocols and the necessity for handling certain virus strains in high biological containment facilities. In an attempt to address this, avian and human influenza HA pseudotyped retroviruses have been successfully employed in antibody neutralization assays. In this study we have generated equine influenza pseudotyped lentiviruses for serological screening. This was achieved by co-transfection of HEK293T cells with plasmids expressing the haemagglutinin (HA) protein of different H3N8 subtype equine influenza virus strains, HIV gag-pol and firefly luciferase reporter genes and harvesting virus from supernatant. In order to produce infective pseudotype particles it was necessary to additionally co-transfect a plasmid encoding the TMRPSS2 endoproteinase to cleave the HA. High titre pseudotype virus (PV) was then used in PV antibody neutralization assays (PVNAs) to successfully distinguish between vaccinated and non-vaccinated equines. The sera were also screened by single radial haemolysis (SRH) assay. There was a good correlation between the results of the two assays, with the PVNA assay appearing more slightly more sensitive. Future work will extend the testing of the PVNA with a larger number of serum samples to assess sensitivity/specificity, inter/intra-laboratory variability and to define a protective titre.

**POSTER 32**

**Comparison Of The Virucidal Effects Of Disinfectants Against Equine Influenza A Virus**

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Equine influenza (EI) caused by infection of equine influenza A virus (EIV, H3N8) is an important respiratory disease in horses because of its contagiousness. EIV is circulating worldwide with posing an economic impact on horse industries. Elimination of the sources of EIV is the important biosecurity measure during EI outbreaks. As the contaminated instruments including stables, saddles, transportation vehicles, personal clothes, etc. can play roles as fomites, the selection of appropriate disinfectants becomes a key issue. Although there are currently many disinfectants available, the information about their effects against EIV is limited. This study is conducted to evaluate the effects of various commercial disinfectants against EIV. The effects of six disinfectants [three quaternary ammonium compounds (QACs), one chlorine-based compound, one iodine-based compound and Vircon-S] against EIV (A/equine/Yokohama/aq13/2010) were evaluated with the different conditions [reaction time (10/30 min), temperature (4-25°C), absence/presence of organic matter].

Didecyldimethylammonium chloride (QAC) diluted in recommended concentration inactivated EIV at ≥15°C regardless of reaction time and the presence of organic matter. Benzalkonium chloride (QAC) showed the lowest efficacy among the QACs tested. Although the efficacy of sodium dichloroisocyanurate (chlorine-based) and nonoxynol iodine (iodine-based) were unaffected by decreasing reaction time and temperature, their effects were affected by the presence of organic matter. The efficacy of Virkon-S was unaffected by reaction time, temperature and the presence of organic matter.

Disinfection should be an integral of routine environmental hygiene management practice to minimize spread of potential infection transmitted by fomites and contact between horses and personnel. Thus, efficacy, safety as well as fewer discomforts to horses and workers becomes important factors to opt for the disinfectants. QACs diluted in recommended concentrations are generally tasteless, odorless, and virtually non-toxic with some exceptions. Thus, our data suggest that didecyldimethylammonium chloride diluted in warmed water is useful in equine industry. Although Virkon-S was effective regardless of reaction time, temperature and the organic matter, its corrosivity may become constraint on disinfecting metallic instruments such as transportation vehicles.

**POSTER 33**

**Imported Pigs Introduced The First Classical Swine Influenza Viruses Into Mainland China**

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Chinese National Influenza Center, WH0cc

Abstract: Objectives: The first classical swine influenza A H1N1 viruses were isolated in mainland China in 1991. To aid surveillance of swine influenza viruses as part of pandemic preparedness, we sought to identify their origin. Methods: We sequenced and phylogenetically analyzed 19 swine influenza viruses isolated in 1991 and 1992 in China and compared them with viruses isolated from other regions during the same period. Results: Classical swine H1N1 influenza viruses were predominant in Beijing pig herds during this period. Based on data on pigs imported to and exported from China, we concluded that these viruses spread to China via pigs imported from North America and that they could affect the genetic evolution and transmission dynamics of swine influenza viruses in Hong Kong. Conclusions: The transmission of swine influenza virus genes via pig transportation highlights the importance of enhanced swine influenza surveillance—especially of imported and exported pigs—for pandemic preparedness.
POSTER 34

Development Of A Fowlpox Virus-Vectored Equine Influenza Vaccine
Ting Qi, Wenhua Xiang, and Xiaojun Wang* Harbin Veterinary Research Institute, the Chinese Academy of Agriculture Sciences, Harbin, China 150001

China experienced an outbreak of equine influenza during 2007–2008. Meanwhile, its neighbor countries, such as Mongolia, India and Japan, have also been affected by various influenza virus strains. Phylogenetic analysis showed that the newly emerging Chinese strains belong to Florida sublineage clade 2, as well as the Indian strain Jammu-Katra/6/08 and the Mongolian strain Mongolia/1/08.

We developed a fowlpox virus-vectored equine influenza vaccine expressing the hemagglutinin gene of H3N8 equine influenza viruses. Two recombinant fowlpox viruses (rFPV-XJ and rFPV-QH) were constructed to express the HA genes of American lineage virus strain A/equine/Xinjiang/3/2007 and European lineage virus strain A/equine/Qinghai/1/1994. The recombinant viruses expressed proteins could react with equine influenza positive sera by western blot or indirect immunofluorescent assay. In order to identify the efficacy of the recombinant virus as vaccine, we test the candidate in animal models. First, the grouped mice were immunized with different recombinant vaccine or control, and challenged with A/equine/Xinjiang/3/2007 or A/equine/Qinghai/1/1994. The results showed that two recombinant equine influenza vaccines could induce virus specific antibodies and could protect mouse from the challenge of equine influenza virus, while all the control groups were infected by the virus. Second, pony experiment showed that the recombinant vaccine could induce high level antibodies after vaccination and protect animal from disease, while the control group could shed the virus continuously and developed to clinical diseases. In conclusion, the animal experiment study showed that the recombinant vaccine could protect animals from challenge of the virus and has potential application in the prevention of equine influenza infection.

POSTER 35

Prior Infection With 2009 Pandemic H1n1 Confers Protection Against European Swine H1 Influenza Viruses
Yu Qiu, Karen van der Meulen, Kristien Van Reeth.
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Salisburyaan 133, B-9820 Merelbeke, Belgium

Objectives
The 2009 pandemic H1N1 influenza virus (pH1N1) is of swine origin and is now widespread in humans and pigs. Besides pH1N1, avian-like H1N1 and human-like H1N2 swine influenza viruses (SIVs) are the dominant H1 viruses in European pigs and they have the potential to infect humans. It remains unknown to what extent immunity against pH1N1 virus in humans or in pigs could confer protection against European H1 SIVs of different lineages.

Methods
Four groups of 5 pigs were inoculated first with pH1N1 virus. Six weeks later they were inoculated with the same virus (pH1N1-pH1N1), or one of three European H1 SIVs: an H1N1 SIV in which all genes are avian-origin (pH1N1-H1N1), an H1N2 SIV in which the HA originates from human viruses from the mid 1980s (pH1N1-H1N2), and a reassortant H1N1 virus that combines the human-like H1 and the avian-like N1 (pH1N1-H1N1). Three unprimed challenge control groups of 5 pigs were inoculated with H1N1, H1N2, or rH1N1 only. All inoculations were performed intranasally with 10⁷ EID₅₀ of viruses. Nasal swabs for virus titration were collected daily for 7 days after each inoculation and blood for serology was collected at various timepoints.

Results
After the first inoculation, all pigs excreted pH1N1 virus for 6-8 days and developed antibodies to the pH1N1 virus. Before challenge, cross-reactive antibodies against H1N1, rH1N1 or H1N2 SIVs were undetectable in the hemagglutination-inhibition test, but most pigs had low cross-reactive antibody titers in the virus-neutralization test. All pH1N1-inoculated pigs developed neuraminidase-inhibition antibodies against H1N1 and rH1N1 SIVs, reflecting the same NA lineage of these viruses. After challenge, all challenge controls excreted high amount of virus for 5-6 days. Conversely, virus excretion was undetectable in the pH1N1-pH1N1 and pH1N1-H1N1 groups. Partial cross-protection was observed in the pH1N1-rH1N1 and pH1N1-H1N2 groups: virus was isolated from 3 pH1N1-rH1N1 pigs for 1 day and from 4 pH1N1-H1N2 pigs for 1-3 days.

Conclusions
Robust cross-protection against European H1 SIVs was observed in pigs immune to pH1N1 virus, and the extent of protection was correlated with the genetic relationship of the viral HA and NAs. People are likely at a reduced risk for zoonotic infection with European H1 SIVs since the circulation of 2009 pH1N1 viruses.

POSTER 36

An Ex-Vivo Dog Tracheal Organ Culture System For The Study Of Canine Influenza Emergence
1 MRC-University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; 2 Wepers Centre Equine Hendaye, School of Veterinary Medicine, University of Glasgow, UK; 3 School of Veterinary Medicine, University of Glasgow, UK; 4 The National Institute for Medical Research, The Ridgeway, Mill Hill, London, UK; 5 Charles River Laboratories, Preclinical Services, Tranent, Edinburgh, UK

Background: Influenza viruses represent a significant risk to human and animal health. Determining the mechanisms underpinning cross-species transmission is critical in understanding viral emergence. Equine influenza virus (EIV) jumped the species barrier in the early 2000’s and emerged as a novel respiratory virus of the dog, canine influenza virus (CIV). The EIV–CIV system provides a
unique opportunity to study the mechanisms that govern influenza emergence.

Objective: We aimed to determine the evolutionary dynamics of EIV adaptation to the dog respiratory tract. To this end, we developed an ex-vivo organ culture (EVOC) system of dog trachea with air interface.

Methods: Dog tracheal explants with an air interface were prepared and maintained in culture for up to seven days. After 24 hours in culture, explants were infected with a variety of EIVs. Changes in the histology and ciliary function of infected explants were assessed to define a phenotype of infection. Immunostaining techniques were used to identify different cellular subpopulations as well as the target cells of EIV infection. Viral growth was quantified by flow cytometry.

Results: The histological and physiological features of tracheal explants were maintained for up to seven days. Explants exhibited susceptibility to EIV infection and displayed histological changes consistent with those observed in vivo such as loss of cilia and a reduction in epithelium thickness.

Preliminary results show that EIV can replicate in dog trachea and that ciliated epithelial cells are likely to be the target of infection.

Conclusion: The EVOC system of dog trachea constitutes an amenable system for the study of CIV emergence.

POSTER 38
Development Of An Influenza A Sequencing Workflow On Ion Pgm™ Sequencer For Improved Surveillance
A. M. Burrell1, C. Cummings2, W. Ge1, and C. O’Connell1

Background: Detects of swine influenza viruses (SIVs) in humans and human influenza viruses in pigs are increasing. This has been particularly evident since the 2009 H1N1 pandemic virus rapidly spread around the world to man and pigs. Reassortant variants of SIVs are particularly troublesome, especially a H3N2v which has infected more than 300 humans in the United States and is thought to be enzootic in US swine herds. As recent reports have indicated that SIVs may be missed with human diagnostics, we sought to compare US Centers for Disease Control & Prevention (CDC) qRT-PCR, and Quidel Corporation’s (San Diego, CA) commercial influenza diagnostics (Molecular Influenza A, QuickVue Influenza, and Sofia Influenza A assays) in detecting SIV from pig swabs.

Methods: Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) shipped, on icepacks, a blinded panel of 200 pig clinical nasal swab specimens (0.2mL undiluted aliquot each) to the Global Pathogens Laboratory. Collected from 2010 to 2012, 147 of these specimens had been previously identified to be influenza A-positive by molecular assays. In 2010, specimens were tested using a homebrew qRT-PCR assay for influenza A. Beginning in 2011, samples were tested with a USDA-approved veterinary qRT-PCR VetMAX-Gold SIV detection kit (Life Technologies Corporation, Carlsbad, CA).

Results: Compared to the ISUVDL influenza A assays, the CDC and Quidel assays lacked sensitivity but had excellent specificity in detecting influenza A in pig swab specimens.

<table>
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<th>Sensitivity (95% Confidence Interval)</th>
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<td>CDC qRT-PCR</td>
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<td>Quidel Molecular Influenza (qRT-PCR)</td>
<td>0.60 (0.52-0.68)</td>
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Conclusions: While some reduction in sensitivity may be due to dilution of original samples, freeze thaw cycle RNA degradation, and slight changes in RNA extraction and molecular assay protocols, it seems clear that molecular and immunological assays, that are designed for detection of human influenza A virus, may not be equally effective in detecting SIV.

POSTER 37
Evaluation of quidel® influenza assays in detecting Swine influenza viruses
Benjamin D. Anderson1, John P. Burks1, Gary L. Heil1, Hai Hoang2, Jianqiang Zhang2, Kyoung-Jin Yoon2, and Gregory C. Gray1

1College of Health Professions and Global Pathogens Laboratory of the Emerging Pathogens Institute, University of Florida, Gainesville, Florida 2Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa

Background: Detections of swine influenza viruses in humans and human influenza viruses in pigs are increasing. This has been particularly evident since the 2009 H1N1 pandemic virus rapidly spread around the world to man and pigs. Reassortant variants of SIVs are particularly troublesome, especially a H3N2v which has infected more than 300 humans in the United States and is thought to be enzootic in US swine herds. As recent reports have indicated that SIVs may be missed with human diagnostics, we sought to compare US Centers for Disease Control & Prevention (CDC) qRT-PCR, and Quidel Corporation’s (San Diego, CA) commercial influenza diagnostics (Molecular Influenza A, QuickVue Influenza, and Sofia Influenza A assays) in detecting SIV from pig swabs.

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genomic fraction covered by reads significantly improved when the PathAmp™ reagents were used.

Results obtained - Mean coverage depth was approximately 700-fold higher and host genome contamination dropped from >26% to <0.25% with the PathAmp™ reagents. Sensitivity of the PathAmp™ reagents was tested by serially diluting swine influenza virus in porcine nasal swabs and tonsil tissue. Lineage calls for both sample matrices were correct down to 200 viral copies demonstrating the sensitivity of this technique.

Conclusions - The PathAmp™ FluA Reagents provide a rapid, accurate, and sensitive solution for Influenza sequencing on the Ion PGM™ allowing for faster responses to emerging strains.
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