Summary of the OFFLU Swine Influenza Virus Group Meeting 2013

FAO Headquarters, Rome, 16 - 17 April 2013

Participants: Amy Vincent (USA), Ariel Pereda (Argentina), Chuanling Qiao representing Hualan Chen (By teleconference - China), Clement Meseko (By teleconference - Nigeria), Filip Claes (FAO), Frank Wong (Australia), Gounalan Pavade (OIE), Gwenaelle Dauphin (FAO), Ian Brown (UK), Janice Ciacci Zanella (Brazil), John Pasick (Canada), Keith Hamilton (OIE), Kristien Van Reeth (Belgium), Liz Mumford (WHO), Malik Peiris (By teleconference - Hong Kong), Marie Culhane (By teleconference - USA), Nicola Lewis (UK), Bandit Nuansrichay (Thailand), Richard Webby (USA), Ruben Donis (USA), Sabrina Swenson (USA), Steve Edwards (UK), Taki Saito (Japan), Tung Nguyen (Vietnam)

Day 1 – April 16

Dr Lubroth, Chief Veterinary Officer, FAO welcomed the participants and wished for a fruitful meeting. He acknowledged the importance of swine surveillance worldwide and sharing the data which benefits both public and animal health.

Current status of swine influenza virus (SIV) by region and activity updates

Canada (John Pasick):

The Canadian swine population has remained steady at approximately 12 million pigs, however, 2012 was a difficult year marked by significant financial hardship. To illustrate this, Big Sky Farms of Saskatchewan, Canada's second pig producer and Puratone Corporation of Manitoba, Canada's fourth largest pig producer, both filed for bankruptcy in September 2012. In the period 2009 to 2010 nine laboratories across Canada conducted influenza A virus testing on 2993 submissions totaling 15,937 swine origin samples. The National Centre for Foreign Animal Disease in Winnipeg is collaborating with provincial veterinary diagnostic laboratories in Quebec, Ontario and Manitoba to genetically and antigenically characterize SIV isolates from 2010 to present. From 2010-2012 72 SIVs were isolated, whole genome sequencing and antigenic characterization has been performed on 26 SIVs. A variety of reassortants with A(H1N1)pdm09 virus have been identified on the small number of viruses that have been characterized to date. Antigenic characterization of isolates will be done using a panel of reference antisera obtained from Dr. Amy Vincent. Attempts are underway to obtain funding to characterize SIV isolates over the next 3 years as well as to design a prototype national integrated surveillance system for zoonotic influenza viruses in Canada. In the public health realm there have been no reports of H3N2v infections of humans although there was a single case of a person from Ontario infected with a gamma cluster H1N1 virus in September 2012. This individual had worked with pigs in Canada and the USA so the source of infection is uncertain.

USA surveillance (Sabrina Swenson, Marie Culhane):

The USDA SIV surveillance program is an opportunity to monitor changes nationally. University of Minnesota is a participant in USDA surveillance program but also receives samples through private veterinary practitioners, outside the scope of the USDA system. Samples from sick pigs, pigs related to public health investigations of novel flu cases, and swine exhibiting influenza like illness at exhibition events are routinely submitted. In 2012 there was a dramatic increase in samples primarily due to a greater willingness to participate.

From October 2009 to January 2013, a total of 23953 samples were tested in the USDA system, of which 5498 were positive for matrix gene by PCR. The predominant subtypes were H1N1, H1N2 and H3N2 with a minor but consistent detection of mixed subtypes. The National Veterinary Services Laboratory (NVSL) has a repository of around 1600 SIV. Full genome sequencing is done for representatives of each subtype H1N1, H1N2 and H3N2 and for representative viruses of each submitted state.

At the human-animal interface, samples are screened by PCR and sequencing to feed information to the State/Federal animal health and public health agencies. Human infections with H3N2v increased to 309 in 2012 compared to 12 in 2011.

USA research (Amy Vincent)

- Swine IAV were analyzed by subtype using time series decomposition 2009 – 2012. Overall the virus infection exhibited a seasonal pattern with two peaks. The first peak occurred in late November-December, the second one in March-April. H3N2 and H1N1 increased in abundance, whereas H1N2 remained steady. Although it is difficult to predict what is coming next, control strategies do not seem to have been effective and should be reassessed.

- H1 phylocluster time series abundance 2009-2012 indicated A(H1N1)pdm09 has declined to only sporadic detections; Delta1 and Gamma H1s remain frequently detected and Beta H1 although detected less frequently, were slowly increasing during the study period[1]. This waxing and waning of cluster types suggests possible introduction of these phylotypes by pig movement within the US and from Canada to the US, loss in vaccine efficacy or ineffective application of vaccine, and/or waning herd immunity to the drifting viruses (T. Anderson, submitted).

- The H3 phylogenetic analysis showed 6 subclusters of H3 (A to F) emerging from Cluster IV. Cluster IV-A included most of the H3N2v human isolates and most H3N2v from 2011-12 were genotype 1. (Kitikoon *et al*. J Gen Virol, 2013, 94, 1236-41). 10 H3N2 genotype patterns of contemporary reassorted H3N2 (rH3N2p) virus have been identified in U.S. Swine since 2010.

- The phylogenetic analysis of N2 sequences demonstrated two clusters as published (Nelson M I *et al.* J. Virol. 2012; 86:8872-8878). The two major clades on N2 tree are those related to the original

[1] In the US, SIVs are designated to several clusters based on genetic similarity. Phylogenetic analyses of HA genes illustrated the evolution of multiple cluster types (alpha, beta, gamma, delta and pandemic) from viruses in USA swine populations. For more information refer Lorusso A, Vincent AL, Gramer MR, Lager KM, Ciacci-Zanella JR. Contemporary Epidemiology of North American Lineage Triple Reassortant Influenza A Viruses in Pigs. Curr Top Microbiol Immunol. Jan 22, 2012

triple reassortant SIV identified in 1998 and a newer cluster most closely related to human H3N2 influenza viruses collected in 2002.

- Vaccination studies showed maternal antibody interfered with vaccine protection and live attenuated vaccine is the only vaccine that prevents shedding/transmission of contemporary H3N2.

USA NIH Centers of Excellence in Influenza Research and Surveillance (CEIRS) (Richard Webby)

Active surveillance was done on 32 selected farms in Illinois, Indiana, Iowa and Minnesota. A total of 16170 samples were collected between June 2009 and December 2011. 746 (4.6%) nasal swabs tested positive by real time RT-PCR and 178 viruses were isolated. Seasonality was not noted and mostly 1 to 5 swabs positive out of group of 30. Basically there was no difference in the types of virus based on sick pig detection (USDA swine surveillance) versus the targeted surveillance at farms of healthy animals.

By logistic regression analysis, farm risk factors such as farm type, pig flow and gilt source were significantly associated with levels of influenza virus detection while sow vaccination, number of barns on-site, barn ventilation, number of pigs within a mile, number of employees, presence of other animals at the farm were not associated.

US Public health, Centers for Disease Control and Prevention (CDC) (Ruben Donis)

The public health opportunities for OFFLU SIV group includes sharing of global surveillance data, assessing risks by virus characterization and supporting development of effective responses by diagnostics and vaccination. The lessons learned from A(H1N1)pdm09 showed influenza will continue to be unpredictable and early detection of pandemic threats with rapid development and testing of vaccines is necessary. To strengthen risk identification and assessment, CDC has developed an Influenza Risk Assessment Tool (IRAT) comprising 10 elements (Trock *et al.,* Avian Diseases 2012, 56, 1958-61). The OFFLU experts contributed to various elements like receptor binding, genomic variation, animal models transmission, global distribution, infection in animals and antigenic relationship. A win-win result could be achieved by intersectoral collaboration among public health sector goals and animal production and animal health sector goals.

- A document on measures to minimize influenza transmission at swine exhibitions was developed by collaborative efforts of subject matter experts at the Centers for Disease Control and Prevention, the Council of State and Territorial Epidemiologists, the National Association of State Agriculture Officials, the National Association of State Public Health Veterinarians, and the United States Department of Agriculture. http://www.nasphv.org/Documents/NASAHO-NASPHV-InfluenzaTransmissionAtSwineExhibitions2013

Brazil (Janice Ciacci Zanella)

The presentation focused on results of the research project: “Diagnostic, molecular characterization and pathogenesis studies of infectious agents economically important for the Brazilian Swine Production”. The project was conducted at Embrapa (Brazilian Agriculture Research Corporation) and financed by CNPq. The objective of this work was to perform and implement diagnostic methods for economical important infectious agents for swine production, such as influenza A virus (IAV) in

swine. Results presented were from a) samples collected from 16 farrow to finish farms (FF) from 2009-2012 (CNPq project), b) samples from 49 commercial nursery farms 2011-2012 from 7 Brazilian states (commercial farms) andc) samples from Diagnostic Lab (CEDISA - Animal Health Diagnostic Laboratory) totaling 86 lung samples (diagnostic samples). Samples included nasal swabs (NS), serum, oral fluid (OF) and lung tissue. The analyses performed included qRT-PCR, ELISA IAV NP, subtyping RT-PCR, genome sequencing, viral isolation, HI, histopathology (IHC). Serology results from CNPq farms indicated a variation of titers and frequency of anti-IAV antibodies. However, the average frequencies in suckling (63%), nursing (45,2%), growing (58%) or finishing (69,4%) phases indicated the dynamics of infection and circulation of IAV in all phases. HI results showed that H3N2 and A(H1N1)pdm09 were the predominant subtypes on those FF farms. IHC on lung tissues collected at slaughther houses indicated high incidence of *Pasteurella multocida* as secondary agent. Serologic investigations of 49 farms from 7 Brazilian pork producing states showed a high percentage (above 60%) of ELISA positive among growing swine (8 – 12 week old pigs). HI results showed highest antibody titers for H3N2, followed by H1N2 and A(H1N1)pdm09. Detection of IAV in NS and OF by qRT-PCR on 62 swine farms (FF and comercial nursery farms) indicated a global concordance of NS pools and OF of 82.26% for the two tests and the Kappa index was 0.613. The sensitivity of real-time PCR for IAV and A(H1N1)pdm09 was 66.67% for OF and 57.69% for NS, whereas specificity was 92.11 for OF and 100% for NS. Viral isolation was performed on lung and NS samples and resulted in 50 IAV isolates (3 from NS and 47 from lungs). Subtyping of HA and NA on 28 samples detected H1 (11 samples), H3 (1) and N2 (14), most of the samples were untyped. Diagnostic samples (2009-2012) analysed 86 lungs for screening of respiratory agents involved in the porcine respiratory disease complex (PRDC). IAV was the most frequent agent (65% of the lungs). Genome sequencing data for HA, NA and M was performed on 26 IAV isolates. HA sequencing grouped most of the Brazilian sequences in the pandemic cluster with 98-99% identity, with the exception of one H1 (delta cluster) sequence. NA sequencing grouped most of the Brazilian sequences in the pandemic N1 cluster, with the exception of one N2 (human-like) sequence. All Matrix gene Brazilian sequences grouped with the pandemic cluster with 98-100% identity.

Central and South America (Ariel Pereda)

In Central and South America, reports of 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 to be determined.

In Colombia, 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 was carried out on 78 herds from the three major swine rearing areas of Colombia with an estimated prevalence of almost 50%. Nasal swabs, lungs and bronchial aspirates were processed for virus isolation. Overall serological reactivity by HI test was 69.0% for H1 and 49.3% for H3. Fifteen strains isolated from 9 herds from 3 regions evaluated were analysed, 12 of them corresponded to A(H1N1)pdm09 and 3 to H1N1 classical strains.

In Chile in 2009 a number of swine production sites experienced coughing in the maternity and raising units. This correlated with an increase in respiratory disease and greater weight loss of the animals, ultimately affecting productivity. A serological survey performed on 13 production sites

revealed an overall seropositivity of 48% to H1N1 strains and 22% to H3N2 strains. Positive animals were found in basically all the production sites and units (e.g. maternity, reproduction, raising units, etc.) demonstrating widespread infection. The subtypes isolated and characterized in Chile were classical swH1N1, swH3N2 and A(H1N1)pdm09. Those subtypes continued to circulate in the last 3 years, thus producers have begun vaccinating sows (during the gestation and maternity period) with an autogenous vaccine. Vaccination has led to decreased coughing and clinical symptoms, an overall weight gain of the animals, a substantial decrease of the cost of treatment of respiratory illnesses and reduced mortality.

In Guatemala a two-year (2010-2011) cross-sectional study for the detection of A(H1N1)pdm09 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 positive samples were observed in 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 on the border between Guatemala and Honduras, around the capital, Guatemala City.

In Argentina the circulation of SIV in pigs was initially explored by retrospective serology using ELISA showing a prevalence of approximately 41%. Wholly human H3N2, A(H1N1)pdm09 (Argentina reported the second case of the A(H1N1)pdm09 in pigs in the world), and novel SIVs derived by independent reassortment events were reported. Thereafter 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. The subtypes isolated in Argentina are a wholly human H3N2, A(H1N1)pdm09 and reassortants between the A(H1N1)pdm09 internal genes and HA and NA from H1N2 (delta 2) and H1N1 (delta 1) and the human H3N2. At this stage, it is not known whether reassortment among SIVs in Argentina is a common occurrence and/or reflects the exponential growth of the swine industry.

In conclusion SIVs circulate in pigs in Central and South America and there is a year-to-year variation suggesting that levels of influenza transmission may vary along the year. Mostly the viruses isolated have a human origin and the principal subtype is the A(H1N1)pdm09 virus. These viruses started to reassort between them, at least in Argentina.

It will be helpful if a SIV reference laboratory for South and Central America is established, because there are some issues that hinder the shipment of samples to the current reference laboratory in USA (Ames, IA), as the presence of certain diseases in the region (FMDV and/or CSFV) make it difficult to ship samples and finally characterize the disease.

<u>European swine influenza network (ESNIP) (Ian Brown)</u>

The ESNIP project is in the third year of funding. The project consortium consists of 25 partners and vaccine manufacturers. There have been 1533 positive cases out of 4413 herds investigated in UK, Belgium, Netherlands, France, Italy, Denmark, Poland, Slovakia, Spain, Germany, Finland, Israel, Hungary and Greece. Of these 53% belonged to the avian-like swine H1N1 lineage (introduced in 1979), 16% belonged to the human-like reassortant swine H1N2 lineage (introduced in 1994), 9% belonged to the human-like reassortant swine H3N2 lineage (widespread since 1984), 8% were

reassortants between the enzootic H1N1 and H1N2 lineages, and 14% were A(H1N1)pdm09 -like viruses or reassortants that have acquired gene(s) from A(H1N1)pdm09. 60 full genomes from 11 countries were genotyped. Four types of genomic variation (Avian–like H1N1, H1N2, H3N2, A(H1N1)pdm09) were detected in European SIVs.

Europe research (Kristien Van Reeth)

FLUPIG www.flupig.ugent.be is a Framework 7 Program project funded by the European Commission (July 2010 - December 2014, approx. 5 million euros) consisting of 10 international partners. The aim of the project is to gain insights into the role of pigs in the overall influenza ecology and specifically in the generation of human pandemic viruses. Avian H9N2, avian-like swine H1N1 and A(H1N1)pdm09 are being used to study what makes avian influenza virus adapted to pigs, what determines transmission between pigs and from pigs to other relevant species. The ferret is used as a model for human transmission. Attempts to unravel the genetic determinants of avian influenza virus adaptation to pigs are made by a) serial passages of wholly avian viruses in pigs combined with sequencing of seemingly swine-adapted viruses, b) investigations of reassortants between avian and swine adapted viruses for their replication in and transmission between pigs. Other studies focus on the distribution of Sia alpha 2-3 versus Sia alpha 2-6 receptors in the porcine respiratory tract, and on the significance of the receptor-binding preference of influenza viruses for replication efficiency and transmission in the pig.

China - Chuanlin Qiao (for Hualan Chen)

- A biannual surveillance program was conducted on SIV during 2012-2013. Around 12000 nasal swabs from 20 provinces were tested. A total 76 SIVs were isolated from these samples, including 50 H1N1 SIVs, 25 H1N2 SIVs, and one H9N2 SIV. The H1N1 includes avian-like H1N1, classical H1N1 and A(H1N1)pdm09. Research findings indicated novel reassortments happened between 2009 pandemic and endemic H1N1 or H1N2 swine influenza virus.

- As part of an international cooperation project on swine influenza surveillance, currently 10000 nasal and serum samples were collected from pigs in Guangdong and Hunan provinces and diagnosis are underway.

- Following human infections with H7N9, 2150 nasal swabs were collected from pigs from six provinces during early April 2013. No influenza virus was isolated from these samples.

Hong Kong (Malik Peiris)

- Abattoir surveillance of pigs in Hong Kong from 2007-2012 showed increased isolation rates of SIV during recent years. A total of 314 isolates were obtained from 25010 samples collected over six years.

- HI tests on 260 swine sera collected in 2011-2012 revealed around 60% seropositivity to any H1.

- An increase of SIVs with A(H1N1)pdm09 genes was detected. In 2012 75% of the reassortants had genes from A(H1N1)pdm09 and 25% had no A(H1N1)pdm09 genes. The latter viruses only had Eurasian or TRIG genes.

Sri Lanka (Malik Peiris)

- 5420 nasal and tracheal swabs and 1773 serum samples were collected from August 2009 to May 2012. 26 numbers of A(H1N1)pdm09 like viruses were isolated.

- A(H1N1)pdm09 influenza virus activity in humans and swine in Sri Lanka (2009-2012) indicated repeated spill over from humans to swine without long-term establishment in swine.
(Perera *et al* Emerg Infect Dis 2013; 19 (3); 481-484)

Thailand (Bandit Nuansrichy and Taki Saito)

Four national surveillance schemes are underway. It includes national surveillance in Thailand using virological and serological surveillance (2010-2012), collaboration with National Institute of Animal Health (NIAH, Japan) for surveillance of healthy pigs (2007-2015), Armed Forces Research Institute of Medical Sciences (AFRIMS, US) for human-animal interface SIVs infection and the Thai ministry of public health sero surveillance (2010-2013).

- In the period 2010 - 2012 surveillance activities were done in 15 provinces covering 24 farms and 900 samples were collected. Four viruses were isolated from routine diagnosis process at NIAH. Serosurveillance using HI test indicated circulation of H1N1 and H3N2 SIVs.

- Through the collaboration project with Japan, 14 viruses were isolated from 840 nasal swabs in 2012 and 2013.

Japan (Taki Saito)

SIVs were isolated from three provinces with H1N2 in Tochigi (2012), H1N2 in Mie (2012) and H3N2 in Miyazaki (2013). The H1N2 has classical HA, human-like NA and A(H1N1)pdm09 internal genes. The H3N2 has human like H/N with A(H1N1)pdm09 internal genes.

Vietnam (Tung Nguyen and Taki Saito)

The surveillance programs in Vietnam include Department of Animal Health (DAH, Vietnam)-NIAH (Japan) cooperation in 2009-2012, EPT plus (DAH-FAO/USAID) and DAH-CDC Co-ag. The DAH/NIAH SIV surveillance has been conducted since 2010. In 2012, the 6^{th} and 7^{th} rounds of sampling were conducted in some provinces in the north and the south of Vietnam. The samples were collected from 2 types of farm: intensive and family types. There were 1600 nasal swabs sampled and tested and 73 isolates of SIV were isolated in 3 provinces (1 in the north and 2 in the south) which are H1N1, H1N2 and H3N2. The DAH/NIAH surveillance will be continued in 2013 with 2 rounds (July and October/November)

- In Dec 2012 – Jan 2013, the EPT plus surveillance was started. The preliminary result showed 26 H3N2 viruses were isolated from 2 out of 5 farms which had been sampled. The viruses were isolated in floating MDCK (The floating MDCK method was transferred from Japan NIAH by Dr Takemae). Besides, the real time PCR was also utilized and showed the similar result. The EPT plus surveillance are still being conducted in Vietnam.

Australia & Oceana (Frank Wong)

Influenza A in pigs in Australia is nationally notifiable. Currently no active surveillance is in place for influenza A in pigs due to the absence of a nationally agreed surveillance plan, leading to a knowledge gap. The first confirmed detections of influenza infections in Australian pigs occurred in 2009, with the A(H1N1)pdm09 virus introduced from human to pig transmissions. The A(H1N1)pdm09 lineage has since remained in apparent circulation in Australian pig populations. Recent investigations of respiratory disease in commercial pigs in Western Australia (July 2012) and Queensland (August 2012) have identified novel reassortant influenza A viruses in lung tissue and nasal swabs. Characterisation of clinical samples and virus isolates have confirmed independent H1N2 and H3N2 reassortant viruses (in Western Australia); and H1N2 reassortant viruses (in Queensland) with genes derived from old human seasonal influenza A strains and A(H1N1)pdm09. These H1N2 and H3N2 viruses represent the first report of non-pandemic influenza A virus in Australian pigs. Molecular evidence supports the probable long-term circulation of human-derived H1 and H3 viruses in Australian swine, previously unsampled due to the absence of influenza A surveillance in pigs. The viruses in Australian pigs are not the H3N2v and H1N2v variant viruses currently circulating in North American domestic pigs that caused concern to public health due to incidences of pig-to-human transmissions in 2011-12.

Sub-Saharan Africa (Clement Meseko)

SIV is an emerging disease in Africa. Pigs are widely kept in the region but receive less attention compared to poultry. Current swine population in Africa is 32 million - about 10 million representing 1/3rd in Nigeria (FAOSTAT, 2011: http://faostat.fao.org). Pigs are reared virtually in all countries in Africa, including Egypt. Data on SIV in sub-Saharan Africa is scanty. As of April 2013 only 2 deposits of SIV in GenBank from Africa - Cameroon (free range) and Nigeria (intensive farm) - are found. Retrospective and prospective examination of data in Africa is important. 99.9% of all livestock losses in sub-Saharan Africa are never registered in official disease reports. As of February 2012 A(H1N1)pdm09 was still detected in pigs in Nigeria. 89 (29.4%) of 302 sera from south west Nigeria analyzed by ELISA were also positive for influenza A. HI test showed 97.7% pandemic H1 and 2.3% H3. Surveillance is needed in commercial piggery, human-animal interface, free roaming/backyard pigs and live pig markets. On the other hand, serological and virological investigations for SIV in Côte d'Ivoire, Benin and Togo in 2006-2008 were completely negative (Couacy-Hymann et al., Emerg. Inf. Dis. 2012, 18, 1446-52). In conclusion, many subtypes of SIV are probably circulating in Africa but are yet to be fully described. Virological and serological surveillance of SIV in the region is ongoing.

Global antigenic cartography (Nicola Lewis)

- From the National Animal Disease Center (NADC) preliminary dataset there are currently 2 major H1 antigenic groups in the US. However there are antigenic variants that do not cluster within these groups and work is ongoing to ascertain whether they represent other antigenic clusters by increasing the size and representativeness of the dataset

- In general for both H1 and H3 subtypes, there are strong correlations between phylogenetic and antigenic clustering. However, there is evidence particularly in H3, that strains that are phylogenetically similar (when coloured by clade) don't always cluster together antigenically.

Investigations on the likely molecular basis for these differences in the limited dataset held are underway. Work is ongoing to increase the dataset to ensure it is capturing as much antigenic diversity as possible, both within the US, and globally. In parallel, integration of HA sequence data into the analyses to determine the amino acid substitution basis for the differences is planned. One example is a strain from Nebraska in 2012 which differs only at amino acid position 145 from others and is antigenically more similar to human seasonal H3 strains from circa 1995.

- It has been demonstrated that some of the amino acid positions that likely resulted in significant antigenic differences among swine influenza H3 viruses are similar to those defined in influenza viruses circulating in other species. E.g. 145 and amino acid 189 in equine influenza. Work is ongoing to determine the molecular basis of the antigenic variation observed in swine strains.

- For both H1 and H3 there is some spatial component to the antigenic clusters that co-circulate. Hong Kong and perhaps other parts of SE Asia have antigenic clusters of their own. Preliminary phylogenetic analyses of global swine influenza H1 datasets showed that clades only appeared to contain strains from particular geographic regions, demonstrating spatial separation of a diversity of swine viruses. This highlights the need to antigenically characterise a representative set of strains from a wide geographic region, without which the full antigenic diversity of SIV currently circulating globally and thus the potential risk to pigs of incursion of SIV from other geographic areas cannot be fully assessed. In addition there is a need to harmonise the definition and nomenclature of a 'clade' to enable comparison of HA data within and among regions and to help inform both the animal health and the public health sectors.

- NADC data suggests that there has been recent significant antigenic drift among the delta-1 strains and there are anecdotal reports that some currently circulating swine influenza strains in the EU are antigenically distinct from other previously circulating strains.

Day 2 – April 17

Dr Steve Edwards, Chairman of the OFFLU Steering Committee opened the day by welcoming and congratulating the group on the amount of work and science accomplished. He briefed the group about the historical background, aims, objectives and specific outputs of the OFFLU network.

International organisations:

FAO updated the progress of the second year of the EPT+ project. The project focused on understanding the role that livestock plays as potential reservoirs for pandemic disease threats and started initially on influenza. Vietnam, China, Thailand and Bangladesh are the countries covered under this project. A total 6165 samples from Bangladesh including 1643 from ducks and 914 from pigs, and 10000 pig samples from China were collected and submitted to partner laboratories for testing. So far the test results from Vietnam using virus isolation, PCR and serology assays indicated six positive farms in north and south regions. Further testing is ongoing. The conclusion of the project will help in harmonization of approaches, identification of capacity needs, optimization of surveillance approaches, political engagement, and one health integrated approach.

FAO tools that might be useful to the SIV group activities are:

- EMPRES-i: Global animal disease information system that includes disease tracking, analysis models, surveillance models and genetics models http://empres-i.fao.org/eipws3g/. The genetic module linking the EMPRES-i data and virus information from open flu database was released recently. The group will be approached soon to have feedback.
- GLIPHA: Global Livestock production and health atlas (agro-ecological data, population data, production data, updated maps) http://kids.fao.org/glipha/
- Risk Modeling and Risk Assessment

OIE: Currently SIV is not an OIE listed disease because it does not fulfill the criteria set for inclusion of a disease in the OIE list. However when non-listed influenza viruses meet the OIE criteria for a '*new and emerging disease'* they are notifiable to OIE; this was the case with occurrences of the H1N1 pandemic virus in pigs. In 2012, the OIE has a global network of 236 Reference Laboratories with 176 experts covering 112 diseases/topics in 37 countries, and 41 Collaborating Centres covering 38 topics in 22 countries. Swine influenza reference laboratories are situated in Italy, Japan, UK and USA. Guidelines for applicants for OIE reference laboratory status are available at http://www.oie.int/en/our-scientific-expertise/reference-laboratories/guidelines-for-applicants/

WHO: There are still gaps on the prevalence/distribution of SIV subtypes with respect to antigenic relatedness to human seasonal viruses and the role of pigs in the influenza risks at the human-animal interface. The public health risks from influenza viruses circulating in swine are unknown. The factors which constitute increased public health risks are field effects of sequence mutations and markers, limited antigenic and global baseline surveillance data in humans and animals. Information regarding referring subtypes and associated diseases in animals and humans publicly caused by SIV is not consistent. Referring to subtypes and reassortants in a valid and standard way among scientists is needed for public heath to interpret swine data. The H5 standardised nomenclature by clades for avian influenza could be a useful way to follow and adopt for SIVs.

Contribution of SIV data to the WHO Vaccine Composition Meeting (VCM)

The OFFLU SIV group contribution to preparedness for biannual WHO vaccine composition meetings was discussed. Based on a June 2012 concept paper, the group worked out the following points for contribution as a starting process.

1) An overview of major virus lineages that circulated in different geographic areas (within the past 5 years and during the past year) and the suggested nomenclature for these lineages. These viruses could be shared for use in human serology studies, development of key reagents, testing of diagnostic assays, and potential vaccine virus development.

2) Current status of genetic and antigenic characteristics of recent virus isolates from swine and changes that may impact diagnostic detection or immunity.

3) Phylogenetic information on available HA and NA gene segments.

4) Any information on viruses associated with unusual epidemiological situations, e.g. increase or change in disease profile, evidence of involvement of other species.

The above information would most likely be compiled yearly instead of twice a year. The information could be provided as a data package or if needed, an attendee from the OFFLU SIV group would be invited to present the data. The OFFLU secretariat will contact WHO to ask for feedback on the concept note provided earlier and work out the modalities of presentation and participation.

A standardized system for cluster and lineage designation for influenza viruses in pigs:

As a point of reference for determining a system for IAV in swine, the group reviewed the WHO, OIE and FAO established guidelines for nomenclature of highly pathogenic avian influenza H5N1 Asian clade divisions based on the molecular sequence of the HA gene. Through the use of standard phylogenetic methods applied to the HA gene, avian H5N1 viruses were effectively grouped into virus "clades" based upon:

1) Sharing of a common node in the phylogenetic tree;

2) Monophyletic grouping with robust statistical support (i.e., bootstrap value of ≥60);

3) Average percentage pairwise nucleotide distances between and within clades of >1.5% and <1.5%, respectively. It is important to note that this cut off is likely too low for SIV.

For SIV, additional criteria as below could be considered for HA.

- Minimum number of isolates within the clade
- Minimum time period of circulation

The situation is more complex with SIV and would need to cover a greater diversity and range of viruses. A working group was assembled and members will include Ian, Amy, Sabrina, Ruben, Nicola and Richard. This subgroup will put forward information for the larger group for review to define specific criteria before submitting the group's recommendations to OFFLU governance and/or the Tripartite.

Following the H1N1 pandemic, the Tripartite of health organisations (WHO, FAO, and OIE) have agreed to develop a system to provide a common name for emerging influenza viruses at the human animal interface (and for other emerging diseases at the human animal interface) which suits their communication needs and does not have a negative impact on public health or animal health. Any outputs from discussion of a technical name that are relevant to the Tripartite discussion will be communicated to the Tripartite.

Report of progress on action items from the previous SIV group annual meeting:

1. A draft document on SIV group statement was presented by Ruben Donis for agreement. The group commented on the document and agreed on a version for the record and posting on the OFFLU website (Refer Annex 1)

2. A draft concept note on SIV group funding designed for donors was presented by Richard Webby for agreement. The group commented on the document and agreed on the version at Annex 2. The document could be used as a preliminary step to stimulate interest from donors, but more details may then need to be provided. The group agreed to review the document once more and post it on the OFFLU website.

3. OFFLU SIV group paper: Amy Vincent updated the status of the OFFLU SIV group paper. The paper has been accepted for publication in the journal "Zoonoses and Public Health" and the pre-print publication copy was shared with the group.

4. Harmonization of laboratory protocols: Ian Brown the group leader of this activity will circulate a questionnaire to the group to collect the information.

5. Lists of Reference Viruses and Diagnostic Tests by Region (Marie Culhane): The group developed a draft document that was discussed in the meeting. The following comments were received to improve the document:

- Make each section consistent in format and content; simplify the list to enumerate separately the contemporary versus historical reference strains; add a contact name to each section

-Focus on isolates with most sequence data available in public database (i.e. whole genome) and viruses available for sharing

- Strive for 2 strains from each lineage, maybe keep one or two historical strains, justify why each virus is in the list (add extra column)

- Focus on isolates to include in phylogenetic analysis, and/or isolates for evaluation of primers/probes for molecular tests, and/or isolates to use in HI

- Generate an "OFFLU" fasta file of HA and NA sequences to easily download from flu database interface such as Influenza Research Database (IRD).

- Include phylogenetic trees for HA and NA that contain these reference viruses

- Viruses from Australia need to be added; as well as viruses from other countries from South America

- Add references for each strain

- Remove serology from document (be more selective and objective)

- Add information on availability of antisera.

6. Reference panel sera:

- Information from the list of viruses and diagnostic tests developed by Marie's group and Nicola data on antigenic cartography could be used to help define the panel and the two groups should work together to make merge the documents into one.
- Exchange sera among laboratories to assess whether a universal serum panel can be recommended for typing swine influenza viruses
- Put together a panel with contributions from North America, South America, Europe, Asia and Australia.

7. Laboratory testing algorithm: Sabrina Swenson presented a draft version of the algorithm. The final version could be posted on the website.

Nomination/voting for new co-chair

- Amy Vincent, the current co-chair of the group stepped down.
- Ariel Pereda was chosen as the new co-chair.
- Kristien Van Reeth will remain as the other co-chair.

One year work plan (2013-14)

- Define 'Reference Panel of Sera' and coordinate activity with 'Regional list of viruses and diagnostic tests' (Ian, Marie, Sabrina, Ariel, Hualan, Frank, Take, Kristien, Nicola): September 30 to prepare and distribute list to group

- Collect regionally specific diagnostic assays, merge with testing algorithm, and OIE swine influenza testing manual (Ian, Sabrina): September 30

- Update Diagnostic Chapter for SIV in the OIE manual (Sabrina): send to group by August 15, comments back by September 15

- Scientific nomenclature for IAV in swine (Amy, Ian, Sabrina, Ruben, Nicola, Richard): first draft proposal to the group by September 1

- Funding note (Richard): group comments by July 15, final version by July 31

- Research Priorities (Kristien, Amy): August 15 to OFFLU SIV group, August 31 comments back to David Swayne and Secretariat

- H1 Global Antigenic Cartography publication (Nicola, Amy, Ariel, John): final HI data by August 31; draft manuscript by September 30

- On-going cross-HI for H1 and H3 antigenic characterization (Nicola, Amy, Frank, Take, Ariel, Janice): serum shared with additional participants by June 30; HI data by September 30

- Update slide set and poster (Janice, Amy, Kristien, Richard, Bandit): working group by August 31; entire group by September 15; completed by September 30

Planning for next meeting:

2014 OFFLU SIV meeting: St Jude CEIRS tentatively has funding to be committed by March 31.

Cost and travel analysis to be worked for the SIV group participation in the following venues and then choose a venue by July 1 to propose to the group

- Ghent University, hosted by Kristien
- INTA, Argentina hosted by Ariel
- EMBRAPA, Brazil hosted by Janice

Standing (L to R): Keith Hamilton (OIE), Bandit Nuansrichay (Thailand), Tung Nguyen (Vietnam), Takehiko Saito (Japan), Ariel Pereda (Argentina), John Pasick (Canada), Janice Ciacci Zanella (Brazil), Richard Webby (USA), Nicola Lewis (UK), Frank Wong (Australia), Sabrina Swenson (USA), Gwenaelle Dauphin (FAO)

Seated (L to R): Ruben Donis (USA), Ian Brown (UK), Amy Vincent (USA), Kristien Van Reeth (Belgium), Liz Mumford (WHO), Gounalan Pavade (OIE)