Summary of the OFFLU Swine Influenza Virus (SIV) Group Meeting
OIE Headquarters, Paris, 3 - 4 December 2015

Participants: Ariel Pereda (INTA, Argentina), Bandit Nuansrichy (NIAH, Thailand), Clement Meseko (NVRI, Nigeria), Joanne Taylor (AAHL, Australia), Gaelle Simon (ANSES, France), Ian Brown (APHA, UK), Janice Ciacci Zanella (EMPRAPA, Brazil), Yohannes Berhane (NCFAD, Canada), Nicola Lewis (U Cambridge, UK), Todd Charles Davis (USA), Sabrina Swenson (USA), Tung Nguyen (NCVD, Vietnam), Gounalan Pavade and Tianna Brand (OIE)

Through Skype - Amy Vincent (USDA, USA), Kristien Van Reeth (Ghent University, Belgium), Marie Culhane (U Minnesota, USA), Bryan Kaplan (St. Jude, USA), Nobuhiro Takemae (NIAH, Japan).

The Director General, OIE opened the meeting by thanking the experts on behalf of OFFLU. He welcomed this initiative to bring together the world's leading experts to coordinate SIV surveillance at a global level and to provide a sustainable platform for sharing and analysing SIV data. He reminded that Zoonotic influenza remains a priority topic for OIE to work with FAO and WHO under tripartite collaborations within One Health concept. With the economic crisis and tightening budgets, he indicated that availability of resources for surveillance is a significant challenge. He supported the approach to make the best of available data gathered through targeted cost effective means, whilst advocating for increased investments in longer term. This provides a strong argument to support the OFFLU SIV group's role in coordinating international efforts. Finally he wished the participants a successful meeting.

The experts presented the current status of SIV by region and recent updates on the research activities of SIV.

Australia and Oceania (Joanne Taylor):

H1N1, H3N2 and H1N2 subtype influenza A viruses (IAV) are circulating globally throughout domestic swine populations and are reassorting continuously with human seasonal IAV strains. This process facilitates mixing of virulence determinants from human and swine origin IAVs which can enable the evolution of a pandemic virus, as demonstrated by the H1N1 pandemic of 2009. In 2012, novel reassortant H1N2 and H3N2 viruses with genetic relationships to older seasonal human IAV were isolated from Australian pigs. The genetic constellations and antigenicity of these viruses are unique among global swine and human IAV. The presence of novel swine IAVs in Australian pigs suggests the potential for commercial herds to act as sources of zoonotic influenza virus transmission. These viruses were characterised in terms of sialic acid containing receptor affinity and infectivity in a range of porcine, human and avian cell lines. The H1N2 reassortant viruses with a pandemic H1N1 2009 backbone indicated possible preferential infectivity of porcine and avian cell lines. The pathogenesis of the novel H1N2 and H3N2 viruses using a ferret model of disease was investigated and showed

typical clinical signs and pathobiology for seasonal human influenza virus infection. Infection by these Australian IAVs in the pig model showed similar histopathology, immunohistochemistry and virus detection in tissues when compared to the ferret model but with no clinical signs. Future work will involve analysis of the of immunology markers in both animal models as well as identification of molecular markers of interest using next generation deep sequencing of animal trial samples.

Japan / South East Asia (Nobuhiro Takemae):

Monitoring of the influenza A viruses of swine (IAV-S) has been ongoing in Japan, Thailand, and Vietnam. In Japan, a swine A(H1N1)pdm09 virus was isolated in 2015. Phylogenetic analysis using previous human and swine A(H1N1)pdm09v revealed the sporadic human-to-pig transmissions, and some of the A(H1N1)pdm09v have been maintained in pig populations. In Thailand, two A(H1N1)pdm09v and 16 seasonal human-like H3N2 IAV-S were isolated in 2015. Phylogenetic analysis revealed the presence of A(H1N1)pdm09v in Thai pig populations that belong to three distinct sub-clades within A(H1N1)pdm09 lineage. In Vietnam, 47 A(H1N1)pdm09v, 75 H1N2, and 153 H3N2 IAV-S were isolated from 2010 to 2015 in active monitoring. H1 HA genes of the Vietnamese IAV-S were phylogenetically divided into A(H1N1)pdm09, classical H1, and pre-pandemic human-like H1 lineages. H3 HA genes were divided into seasonal human-like and triple reassortant H3 lineages. HI test revealed that the antigenicity of the Vietnamese H1, except A(H1N1)pdm09v, and H3 HA proteins differed from the contemporary human H1 and H3 strains, respectively. In fact, a human case of the human-like H3 IAV-S was reported in Vietnam in 2010, suggesting importance of the IAV-S monitoring.

Vietnam (Nguyen Tung):

Since 2009 – 2015, there has been a number of SIV surveillance conducted in Vietnam under the cooperation activities between Department of Animal Health (DAH) of Vietnam and other partners such as Japan NIAH, FAO (EPT+) and USA CDC. Besides, surveillance conducted under cooperation of National Institute of Veterinary Research (NIVR) and CIRAD (France) is also underway.

Among the surveillance programs, the DAH-NIAH surveillance is the first and most updated. In 6 years, there were about 9000 swab samples collected in this surveillance and 275 SIV isolated. These isolates belong to subtypes H1N1, H1N2 and H3N2 and were classified into 16 genotypes based on their gene combination which were pdm09 derivatives, triple reassortant or human seasonal origin. Most of H1N2 and H3N2 viruses possess at least one gene derived from pandemic 2009 H1N1 viruses.

The SIV surveillance activities are continuously implemented to identify hot spots of disease transmission, and better understanding of the viral evolution at the human-animal interface.

Thailand (Bundit Nuansrichy):

In 2013 the swine influenza network laboratory tested 1,610 specimens of which 13 (0.8%) were positive for SIV and includes 8 (1.5%) H1N1 and 5 (0.8%) H3N2. In 2014, a total of 404 samples tested of which 11 (2.72%) were positive only for H3N2 subtype viruses. In 2015, 202 samples tested of which 17 (8.42%) were positive for SIV and includes 15 (7.4%) H1N1 and 2 (0.99%) H3N2 subtype. Some of the SIV isolated this year were characterized and it indicated that both the H1N1 and H3N2 subtype viruses shared similarity with Thai swine influenza isolates in 2013 and 2014.

<u>Africa (Clement Meseko):</u>

Following pandemic H1N1 in 2009, the virus was also detected in pigs in Sub Saharan Africa and has been reported in four countries as of November 2015. The countries include Cameroon, Nigeria, Kenya and Togo. These represent early reports of virological and genomic data on swine influenza from the continent. Thirty percent of over 32 million pigs in Africa are in Nigeria distributed in intensive farms, backyard operations and free range. Trade pigs are also transported in trucks within/outside the country and live pig markets are found in urban zones.

Current surveillance effort targets some of the risk areas in dense pig farm population, live pig markets and slaughter slabs. Samples collected include nasal swabs, lung tissues, and sera. Also, over 2000 African swine fever surveillance samples collected in 7 States are also being examined for swine influenza. Reports of analyses so far showed seroprevalence of 29.4% (Flu A) made up of 98.9% H1pdm, 25.8% H1 and 1.1% H3. Several other studies by scientists in the region also showed positive of 10.4%, 11.8% for flu A viruses.

There are indications of continuous circulation of pdm H1 in a large piggery estate. Other matrix positive samples are still been analyzed for subtypes identification. Virological evidence of classical swine influenza is still being monitored. The potential for circulation and re assortment of pandemic H1 with human seasonal influenza and current epizootics of H5N1 in West Africa is a possibility particularly in poor bio security settings. Yet surveillance is not receiving deserved attention from Governments and Institutions.

Though the population of pigs in Africa is smaller than other region, the continent is however largely at great risk of virus introduction, transmission and spread because of major bio security lapses and should be continuously monitored.

<u>Brazil (Janice Zanella):</u>

Since the emergence of 2009 pandemic H1N1 (H1N1pdm), many outbreaks of respiratory disease were observed in pig herds. The study evaluated influenza A virus (IAV) infection in swine in 48 pig farms located in seven Brazilian states with previous reports of influenza-like signs by clinical, serological and virological cross-sectional studies. Serological results showed that pigs from all farms had anti-influenza antibodies by NP-ELISA. Antibodies to H3N2, H1N2 and H1N1pdm were detected by HI in pigs from 24 farms. Co-infection with two or more IAV subtypes was detected in pigs in seven of those 24 farms. Detection of IAV in nasal swabs and oral fluids by RT-qPCR indicated a global concordance more than 81% for the two biological samples. Moreover, the results showed that H1N1pdm, H1N2 and H3N2 viruses are widespread in Brazilian pig herds.

To characterize the genetic diversity of influenza A viruses in southern and midwest Brazil's large swine population, viral sequencing and phylogenetic analyses on six H1N1 viruses of pandemic origin (H1N1pdm), six H1N2 viruses, and four H3N2 viruses collected in swine during 2009-2012 were performed. Time-scaled Bayesian phylogenies indicated that the genetic diversity of influenza viruses observed in Brazilian swine were derived from multiple previously unidentified cross-species introductions of human seasonal and pandemic influenza viruses. Human seasonal A/H3N2 influenza viruses were introduced into swine twice in the late 1990s in Brazil, and a human seasonal H1N2 virus was introduced in the early 2000s. These human-origin viruses have continued to circulate in

Brazilian swine, exchanging genome segments with each other and with recently introduced H1N1pdm viruses via reassortment (including the pandemic MP segment, which was found in all viruses). A phylogenetic analysis of an additional 28 H1N1pdm viruses collected previously from pigs from South and Central America, indicated that H1N1pdm viruses were introduced into Brazilian pigs from humans at least six times (and possibly as many as 15 times) since 2009.

Argentina, Chile and Guatemala (Ariel Pereda):

In Chile, multiple strains are co-circulating in swine. Four viral strains including SwH3N2, pH1N1-like, SwH1N2, and an H1N2 containing a classical swine Hemagglutinin (cSwH1) and N1 derived from the pH1N1 strain have been identified.

Current analyses suggest multiple introduction of human IAV into swine populations since the late 80's to early 90's and after the 2009 H1N1 pandemic. Additional phylogenetic analyses are ongoing to further characterize the time of introduction and the reassortment events that gave rise to the Chilean swine IAVs

In Guatemala, longitudinal study in backyard pigs was made in between years 2013 and 2014 in two rural communities in proximity to wetlands with migrating waterfowl during winter. Antigenic response against pandemic H1N1 was detected in one pig suggesting IAV interspecies transmission. A cluster of influenza A seropositive households observed may indicate recent influenza virus transmission in this location.

In Argentina there was continuous surveillance for influenza in pig farms. Almost 1400 samples were taken between 2014 and 2015. A(H1N1)pdm09 continue to be the predominant virus and also co-circulate with a H1N2 with an HA from cluster delta 2 and internal genes from the pandemic virus. There was not too much evidence to support reassortment of viruses. There is a serological evidence of continuous circulation of H3 viruses, but in this period no H3 virus was isolated. Vaccination for influenza was practiced in Argentina, but limited.

Europe (Ian Brown):

THE European swine influenza network project (ESNIP 3) According to ESNIP 3 study results there is regional variation in the epidemiology of SIVs in Europe and there are approximately 23 variants detected. ESNIP 3 partner countries Hungary, Belgium, Poland and UK results was presented.

In Belgium, passive surveillance results of 2014-2015 indicated a higher prevalence of av-like H1N1 (41%), followed by H3N2 (36%) and H1N2 (23%). In Hungary, av-like H1N1 remained the predominant virus (75%) followed by pdmH1N1 (25%). In Poland, out of 7 positive cases five were pdmH1N1 and two were av-like H1N1.

France (Gaelle Simon)

In France, passive surveillance of swine influenza A viruses (swIAVs) was first based on research programs and punctual studies but has been reinforced in 2011 by a national program conducted by the Résavip network. This network involves the Ministry of Agriculture, Anses, local veterinary laboratories, veterinarians and farmers. While around 85% of visits are conducted in Brittany, a region containing 60% of the French pig population, sampling is done in almost all regions of

metropolitan France. Swine IAVs are detected in nasal swab supernatants by M gene rtRT-PCR and then submitted to several rtRT-PCRs specific to the different HA and NA genes from European swIAVs for molecular subtyping. Since 2010, more than 1,300 farms have been visited and half were found to be swIAV-positive. Monitoring showed that influenza infection occurred without seasonality in all types of farms and affected all categories of animals, regardless of their physiological stage. A large majority of sampled animals were growing pigs in farrow-to finish operations. The classical epidemiological form affected pigs in all physiological stages equally, while recurrent flu (nearly 40% of cases) was reported preferentially in animals during the post-weaning period (median age of 8 weeks). Passive surveillance enabled the identification of 647 viral strains from January 2010 to October 2015. Almost 68% of the detected viruses belonged to the European "avian-like swine H1N1" (H1avN1) lineage and were circulating in all the regions sampled. "Human-like reassortant swine H1N2" (H1huN2) viruses represented 25.5% of the viruses but exhibited a larger genetic and antigenic diversity than H1avN1 viruses. Furthermore, H1huN2 were identified only in the western part of the country, where reassortant viruses between H1avN1 and H1huN2 were also sporadically identified (3.6%). Whereas "human-like reassortant swine H3N2" viruses were no more isolated in France since 2000', they were detected in 2012 and 2014 in the North, near the Belgian border. Finally, nearly 3% of the cases were found to be infected by the pandemic H1N1 virus (H1N1pdm). Although this proportion remained low at the national level, H1N1pdm circulation seems to have been maintained, but any novel reassortant containing one or more gene(s) from H1N1pdm has been detected to date in France.

Europe research (Kristien Van Reeth):

The European Commission funded "Flupig" research project is now completed. It involved 10 international partners and coordinated by Ghent University. An update was given on the recent research that was initiated during this project, more specifically a) studies on the immune response to influenza virus in swine and novel vaccination approaches and b) studies on the mechanisms of adaptation of avian influenza viruses to pigs. Results of studies with H9N2 avian influenza in pigs were shown. Examples of recent papers by groups outside the Flupig consortium were also given. Refer www.flupig.ugent.be for full information about the project.

Canada (Yohannes Berhane):

The Canadian laboratory is OIE reference laboratory for notifiable avian influenza, however after the outbreak of pandemic H1N1, it has been involved in the monitoring of novel swine influenza viruses in Canadian pigs. The surveillance for the current period was restricted to the 3 main pork producing provinces (Manitoba, Ontario and Quebec) in Canada. For the current surveillance period, 116 clinical samples tested positive on the influenza A matrix based real time RT-PCR assay. Forty nine of these samples were typed as H1 and the remaining 67 samples typed as H3 based on sequencing. Maximum clade credibility phylogenetic tree analysis of the hemagglutinin genes demonstrated that the majority of the H1 viruses belonged to the pandemic H1 (n=45); 2 to beta and 2 alpha clades. The H3 viruses belonged to cluster IV (n=21); IV-C (n=19); IV-B (n=17) and cluster IV-F (10).

Animal experimental studies using novel reassortant EA/NA HPAI H5N1 and H5N2 were conducted in influenza A free pigs. No clinical signs were observed in both groups of pigs. No detectable levels of influenza A virus RNA (Ct values <35) were detected in swab or lung specimens collected from both groups of pigs. Only pigs inoculated with H5N1 were able to seroconvert based on cELISA and

hemagglutination inhibition (HI) assay. No gross or microscopic lesions were observed in the lungs of pigs inoculated with H5N2. Microscopic lesions were observed in the lungs of pigs infected with H5N1 and the lesions were characterized by interstitial infiltration of inflammatory cells, mild bronchiolar changes with degeneration and disorganization of epithelial cells and increased numbers of macrophages in bronchiolar lumens and alveoli. Positive immunostaining for influenza A viral antigen was detected in scattered bronchiolar epithelial cells.

CDC public health update (Todd Charles Davis):

Sporadic human infections with influenza viruses that circulate in swine continue to occur in the United States. While cases have declined in recent years following a high number of cases reported during the summer and fall of 2012, the U.S. Centers for Disease Control and Prevention, in collaboration with the U.S. State Public Health Laboratories, continues to test symptomatic persons. Two cases of A(H3N2)v were identified in the United States in 2015. Direct swine contact was reported in both instances. One patient from Michigan developed illness in June and recovered following oseltamivir treatment. In July, an immunocompromised person from Minnesota developed an acute respiratory illness and tested positive for A(H3N2)v. Virus isolates from each patient belonged to separate phylogenetic groups of the A(H3N2)v hemagglutinin tree. Full genome genetic analysis of A/Minnesota/38/2015 showed this was an H3N2v virus with an HA gene from genetic cluster IV-B. This genetic group includes swine viruses circulating in the Midwest during 2014-2015 and the two recent H3N2v cases from Ohio detected in 2014. Unlike the 2014 H3N2v cases, which both had NP and M genes derived from H1N1 pdm09 viruses, A/Minnesota/38/2015 contained only the M gene from H1N1 pdm09 while the PB2, PB1, PA, NP and NS genes belonged to the triple reassortant swine lineages (similar to H3v viruses detected during 2011-2012 in the U.S). The NA gene was determined to belong to the N2 NA lineage containing other H3N2v viruses and circulating swine viruses. No known markers of resistance to oseltamivir were identified in the NA protein of either the isolate or clinical specimen. Compared to the closest H3N2v candidate vaccine virus, A/Minnesota/11/2010, there were 26 amino acid changes in the HA1 protein. There were three unique HA1 amino acid differences relative to the 2014 Ohio H3N2v viruses and a total of 26 differences relative to the H3N2v CVV. The HI findings indicated that the A/Minnesota/38/2015 and A/Michigan/39/2015 viruses were well-inhibited by antisera to both H3N2v candidate vaccine viruses, A/Minnesota/11/2010 and A/Indiana/10/2011. HI reactivity of each virus to pooled human sera collected post-vaccination with the 2013/2014 vaccine was comparable to other H3N2v viruses as well as a previous human H3N2 vaccine virus, A/Victoria/361/2011. Overall, the data suggested that the human seasonal vaccine could partially protect against these and related H3N2v viruses.

Two cases of A(H1N1)v were identified in the United States in 2015. A fatal case was detected in Ohio during April in a person with potential occupational exposure to swine. A second severe case in Iowa was hospitalized in August. Direct contact with swine was reported. The HA genes of both viruses belonged to the classical swine gamma lineage but were genetically distant to the A(H1N1)pdm09 vaccine virus, A/California/7/2009. Compared to A/California/07/2009 there were 46 amino acid changes in the HA protein. Genetic analysis of the hemagglutinin gene of the H1N1v virus from Ohio (A/Ohio/9/2015) detected the presence of a D222G amino acid change in the receptor binding site (D225G in H3 numbering). Substitutions at this position (D222N or G) have been detected occasionally in H1N1 viruses isolated directly from swine, particularly in viruses of the H1N1pdm09 lineage. In humans, the D222G substitution has been detected in many viruses from severe and fatal

cases of A(H1N1)pdm09 infection, although its public health significance remains unclear and further investigation into the prevalence of this mutation in circulating swine viruses is ongoing. Genetic analysis of Ohio/9 also identified a substitution at position 155 (G155E) within immunodominant antigenic site B. HI reactivity of A/Ohio/09/2015 to pooled human sera collected post-vaccination with the 2013/2014 vaccine was significantly reduced compared to the A/California/7/2009 vaccine virus. Overall, the data suggest that the protection offered by the human seasonal vaccine against this virus would be limited. Thus, per WHO recommendations, generation of an Ohio/9 CVV is underway at CDC. Pathotyping of Ohio/9 in ferret models is underway as are vaccine efficacy studies in ferrets vaccinated with current seasonal vaccines and challenged with Ohio/9.

USA surveillance (Sabrina Swenson):

The USDA influenza A virus surveillance program in swine receive samples from three streams namely sick pick submission, pigs related to public health investigations of novel flue cases and swine exhibiting influenza like illness at events such as auctions, markets, fairs or other swine exhibition events. The surveillance plan was drafted in 2008 and later modified in 2009 to be specific for pdm H1N1 and then broadened in 2010 to account for more subtypes. From 2012 the lab improvised the laboratory algorithm testing methods and in 2014 the Ct cut off values for virus isolation was implemented.

The NVSL holds a repository of around 4400 viruses representing around 30 states. Full genome sequencing is done on random viruses representative of each subtype (H1N1, H3N2 and H1N2) and each submitted state. The lab provides confirmative testing and public health investigations. From 2010 to September 2015, a total of 38350 samples were tested in the USDA system. The predominant subtypes isolated were H1N1, H1N2 and H3N2.

In spring 2015 H3N2 was detected in US dogs. Vet hospital in Chicago area submitted 19 canine and 2 feline samples to NVSL for testing in April. Most animals had a cough and fever unlike the severe, persistent pneumonia seen in Asia with nasal discharge, inappetance, lethargy, sneezing and vomiting. 14 out of 19 canine samples tested positive by matrix PCR and feline samples tested negative. Virus isolation was successful in 8 of 19 samples using embryonated chicken eggs and on sequencing it was found H3N2.

USA surveillance (Marie Culhane):

Between November 1, 2014 and November 30, 2015, the University of Minnesota Veterinary Diagnostic Laboratory performed IAV-S rRT-PCR Matrix Gene tests on 21,467 samples from pigs in 30 US States, 4 Canadian Provinces, 2 Mexican States, and 4 South American countries. One-third of those samples were tested as part of the USDA Voluntary Influenza Surveillance Program for swine. Results of the USDA Voluntary Influenza Surveillance Program are summarized elsewhere. April 2015, July 2015, and October 2015 were the three months with the highest numbers of samples tested. Samples were positive for IAV-S each month, with April and May having the highest the of IAV-S PCR positive results. H1N1, H1N2, and H3N2 viruses were found each month in approximately equal proportions. Only rarely were H3N1 viruses detected. HA gene sequencing of 483 virus isolates revealed the expected genetic diversity, with 6 H1 swine-origin clades and 1 H3 swine-origin clade of influenza A viruses identified. Human-seasonal H1 and human-seasonal H3 clades of influenza A viruses were also identified in the viruses isolated from swine,

albeit rarely. Nevertheless, influenza A viruses of swine are diverse. Human-to-swine transmission, spatial migration via swine movements, and genomic reassortment are the key evolutionary mechanisms that generate this viral diversity, per a 2015 PLOS-Current manuscript by Martha Nelson, Marie Culhane *et al*. Therefore, additional antigenic characterization and whole-genome sequencing is greatly needed to understand the diversity and independent evolution of IAV in swine.

USA research (Amy Vincent):

The USDA National Animal Disease Center is interagency partners with the USDA IAV-S APHIS surveillance system and NIH CEIRS and generates genetic and antigenic analyses and other *in vitro* and *in vivo* studies on swine influenza virus. The USDA IAV-S surveillance for national program activities between Oct 2011 and June 2015 included 85,559 samples. The results include 24,040 diagnostic accessions, 8748 positive accessions, 2898 accessions with viral isolates and 5537 accessions were subtyped.

The HA phylogenetic analyses of the USA summary showed Gamma H1, delta-1 H1 and CIV-A H3 are most predominant nationally. Many internal gene constellations are present in H1 and H3 viruses, the result of reassortment between endemic swine strains and H1N1pdm09 strains.

The HA of human-like H3N2 and H3N1 viruses detected since 2012 are genetically similar to human seasonal H3N2 from 2010/2011, documenting a recent spill over event from humans.

 Swine H1 and H3 antigenic determinants were tested by cross HI assays using standardised swine sera panels. HI data were used to generate antigenic maps to evaluate antigenic evolution along with genetic evolution. A limited number of major antigenic determinants have been identified in H3 HA genes. The delta-lineage H1 viruses demonstrate greater antigenic diversity compared to classical lineage H1 viruses.

Pathogenesis and transmission studies of highly pathogenic avian influenza (HPAI) in the swine host were conducted. HPAI subtypes H5N1, H5N2 and H5N8 from the North American outbreak were used to experimentally infect pigs to evaluate infection and shedding properties. Subsets of primary pigs were euthanized at 3 and 5 days post infection to evaluate replication in lungs, and nasal swabs were collected to evaluate upper respiratory tract replication. Blood was also collected to monitor for sero-conversion. All lungs were positive by RT-PCR, intermittently positive by virus isolation, and all were negative for IAV by immunohistochemistry. Most avian H5 and H7 viruses previously tested in swine failed to sustain efficient replication and transmission, consistent with the U.S. H5Nx HPAI.

A new research project initiative was funded by NIH NIAID CEIRS to systematically assess public health risk of circulating and emerging swine influenza viruses to address pandemic preparedness (Swine-Human Risk Assessment Cross-CEIRS Collaboration). The proposed project is based on utilizing antigenic distances and human population immunity to identify swine IAV of higher risk for further in vitro and in vivo study.

USA NIH CEIRS update (Bryan Kaplan):

The active Influenza virus surveillance of commercial pig populations (2011-2014) in the United States has found juvenile pigs (3 weeks-6 months of age) to have a higher prevalence of influenza A virus compared to gilts (> 6 months of age). Farms housing solely juvenile pigs were found to have

higher odds of influenza infection compared to farms with a mixed population of juvenile and adult animals. In addition, seasonality was observed with increased influenza a virus detection in spring and summer months (February, March, April, May, June). Results from sub-typing analysis show one influenza A subtype to be predominant with detection of multiple subtypes being rare. Genomic analysis of field samples revealed a high level of diversity amongst internal gene segment constellations; mostly combinations of the TRIG and Pandemic lineages. Incorporation of the pandemic MP gene segment occurred with the most frequency (88%), followed by the NP (74%) and PA (59%) segments. Comparison of our active surveillance samples with passive surveillance samples collected by the USDA find both methods of sampling capture a nearly identical representation of influenza A subtypes and internal gene constellations circulating in commercial pig populations in the United States.

Global antigenic cartography (Nicola Lewis):

The global antigenic and genetic analyses paper is under review. The reviewers have requested additional analyses, which work is in progress with a view for re-submission in January.

Led by Amy Vincent and Nicola Lewis there is a new initiative funded by NIAID CEIRS - Determine the public health risk of circulating and emerging swine influenza viruses to address pandemic preparedness (Swine-Human Risk Assessment Cross-CEIRS Collaboration). The proposed project outline and structure of the pipeline was described to experts.

Many surface and internal genes of swine IAV were derived from human seasonal strains and maintain mammalian adaptation properties. Additionally, the current global antigenic diversity of swine influenza viruses creates substantial risks for both human and swine populations. The antigenic divergence of swine IAV to seasonal IAV currently or recently circulating in humans may be a major contributor to the risk of outbreaks and pandemics of swine viruses in humans. This is measurable by assessing the human population immunity to swine viruses. Here, we will determine the public health risk of circulating and emerging swine influenza viruses in global swine populations by a coordinated and systematic pipeline of testing. Swine isolates identified through surveillance and sequencing efforts will be selected to enter the pipeline.

The countries present at the meeting agreed to participate in the prospective initiative by providing strains for assessment. Additionally the EU laboratories (APHA and Anses) agreed to collaborate by antigenically characterizing viruses from other geographic regions, which cannot be exported to the US for exotic disease reasons.

Clade nomenclature activity (Amy /Nicola/Richard/Ian/Todd/Tavis)

A global phylogenetic cluster naming system was proposed based on criteria adapted from avian influenza H5 genetic diversity to be suitable for the global influenza A virus genetic dataset from swine and humans. The naming system was proposed for several reasons: 1) to communicate the genetic relationships between influenza viruses circulating in swine among different geographic regions without incrimination 2) to communicate the genetic relationships between swine and human seasonal influenza viruses. 3) To provide a benchmark to monitor and identify significant genetic evolution of the influenza virus HA gene in the future. 4) To provide a common nomenclature for automated clade tools available on the web, such as IRD (fludb.org).

Draft criteria was proposed and agreed upon. Clades were designated for H1 genes using 6611 unique published sequences for 1A lineage (classical swine), 1B lineage (human seasonal) and 1C lineage (European avian-like) and clade designation for H3 subtype using 1989 unique published sequences for 3A and 3B lineage was presented. It was evident some of the lineages need more data and experts from the concerned regions will be approached for contribution.

The subgroup of experts involved in this task will progress in finalizing a manuscript for submission.

Reference panel sera:

The purpose of this activity was to define a minimum set of sera that would enable the preliminary subtyping of the HA of all strains of SIV from different parts of the world. After careful evaluation it was observed the idea of generating global reference panel of sera is not good and feasible as there are major differences in the SIV between regions and only some similarities exists. It was decided that the laboratories share the list of sera, list of viruses and diagnostic reagents available and post it on the website.

Laboratory testing algorithm:

The pathways used in the laboratory testing of influenza A in swine adopted in NVSL (USA), APHA (UK) and Anses (France) was presented by the concerned experts. It was decided to post a simplified logarithm on the OFFLU website for the use of researchers.

Membership of SIV group members:

The group members discussed about the informal membership of experts to be invited for the annual technical meeting. It was decided to have flexibility to invite new contributing members in consultation with the co-chairs of the SIV group.

Nomination/voting for new co-chair:

Ariel Pereda, the current co-chair of the group stepped down. As many members of the group are absent in the meeting, it was decided to elect the next co-chair through email communications from interested experts. Nicola Lewis remains as the other co-chair.

One year work plan (2016-17)

- A simplified SIV diagnostic logarithm to be developed for posting on the OFFLU website (Sabrina, Ian)

- Laboratories share the list of sera, list of viruses and diagnostic reagents available and post it on the website (all experts)
- Development of new SIV communication materials and poster (Janice, Gaelle, Ariel, Nicola and Ian)

- OFFLU SIV group to explore funding activities through International Research Consortium on Animal Health launched by STAR IDAZ where there are possibilities for many working groups to contribute (Ariel Pereda to investigate and update the group)

- Contribution of influenza data related to swine to human and human to swine in the WHO vaccine composition meetings (Todd Charles to contact WHO)

- A publication will be prepared to share the cluster naming criteria and system publicly. (Amy, Nicola, Tavis Anderson)

Planning for next meeting:

The OFFLU Secretariat will circulate a doodle to all the members to find out a probable month in the calendar of 2016 to hold the next meeting. The expert from Thailand expressed the willingness to host the next meeting in Bangkok. Nicola Lewis will contact Diane Post on the opportunities for funding for the next meeting.

Meeting participants