



## **OFFLU Technical Meeting**

### **Coordinating worldwide surveillance for influenza in pigs**

**OIE Headquarters, Paris, 6-7 April 2011**

#### **Day 1 – April 6**

#### **Objectives and overview of influenza in pigs (*Amy Vincent*)**

- There is an on-going global need for up-to-date knowledge about the incidence, geographic distribution and dynamics of influenza infections in pig populations. A current deficiency is evidenced by a lack of publically available sequences, phylogenetic and antigenic analysis, published papers, research reports, etc.
- There is a need for a concerted global epidemiologic and virological surveillance program for influenza in pigs.
- There are many challenges to global surveillance including:
  - o Access to viruses on farms
    - Producer and veterinary participation is voluntary;
    - Political and regulatory constraints;
    - Producer acceptance of SIV infection is often based on a lack of perceived economic impact on herd health.
  - o Current serologic surveillance techniques are of limited value
    - This is confounded by vaccine usage;
    - and requires knowledge of local circulating subtypes and strains.
  - o Funds are lacking for collection, sequencing, laboratory assays, banking of viruses, generating sera, data management and analysis, etc.
  - o Government infrastructure and programmes for non-reportable disease may not exist.
  - o There is a lack of reference laboratories with swine influenza virus (SIV) expertise in some high priority regions.
  - o International shipping restrictions for biological material due to endemic/exotic diseases.
  - o Restrictions in sharing viruses between laboratories and countries due to a variety of factors including intellectual property rights, fear of sanctions, politics, Material Transfer Agreements (MTAs).

#### **Benefits and drivers for influenza surveillance in pigs (*Kristien Van Reeth*)**

Surveillance for influenza viruses in pigs has been neglected for a long time and is highly needed, but we should also be aware of the limitations of surveillance. For example, the so-called “push button risk assessment strategy” is not realistic, because we don’t know what determines transmission of influenza viruses from pigs to humans or spread between humans. The best use of influenza surveillance in pigs is to provide information about changes in influenza viruses circulating in pigs, to detect novel influenza viruses, and to identify those viruses that can be used for research.

Other benefits of influenza surveillance in pigs include

- supporting vaccine strain selection for manufacture of more effective SIV vaccines;
- assisting with diagnostic reagent design to support more effective animal health/production systems;

- providing a comprehensive scientific evidence base to inform accurate and timely communication and policy;
- virological surveillance allows whole genome sequencing which will support more focused and directed research and is more reliable than serological surveillance;
- a better understanding of the antigenic and genetic evolution of SIVs;
- support to public health efforts to monitor the incidence of zoonotic influenza infections and assess risk to inform pandemic preparedness plans.

### **Current SIV surveillance networks, state of the art in different regions (approach and organization, sustainability, accomplishments, opportunities)**

- **Europe – ESNIP3** (*Kristien Van Reeth*)

The ESNIP programme is funded by the European Commission, and constitutes three 3-yr studies or rather “coordination actions”. ESNIP was established in 2000 in response to a changing global influenza picture in pigs, with the introduction of human-like H1N2 into pigs in Europe, and triple reassortant H3N2 into pigs in the USA. Also, reports of H5 and H9 avian influenza in humans in Hong Kong raised concerns about the potential role of pigs as intermediate hosts.

Achievements of ESNIP1 include

- establishing standardized protocols for diagnosis;
- selection of reference strains and hyperimmune sera;
- establishing a central virus bank and electronic database;
- antigenic and genetic characterization of some recent isolates;
- preliminary picture of the prevalence of different SIV subtypes in different countries.

Achievements of ESNIP2 include

- determining some interactions between animal and human influenzas;
- antigenic and genetic characterization of a (limited) number of European SIVs (Belgium, UK, Italy, France, and Spain): 169 viruses analysed during a 3 year period from 2006 to 2008, including 5 novel reassortants, although the majority were prototype European SIVs;
- serological profiles of fattening pigs to determine infection patterns (Belgium, France, Italy, Spain);
- expansion of virus bank;
- M gene-targeted real time RT-PCR for SIV established.

Goals of ESNIP3 (launched in 2010) include

- whole genome sequencing of influenza viruses isolated from pigs;
- antigenic cartography;
- viruses will be made publicly available at the end of the project.

The most important hurdles for SIV surveillance in Europe are the difficult access to the samples, the fragmented data analysis, and lack of resources and personnel. From the previous ESNIP networks, it was learned that standardisation and harmonisation of protocols and approaches is of crucial importance.

- **USA** (*Sabrina Swenson*)

- Influenza in swine is not a federally reportable disease in the USA.
- Vaccination is permitted and can confound serological surveillance.
- USDA launched a national swine influenza survey in May 2009 with support from CDC.
- Sustainability of such a program will depend on funding and interest by producers and laboratories to continue participating.
- USDA Surveillance for influenza in pigs is targeted at 1) commercial and backyard populations; 2) high potential for human-animal interactions; 3) co-mingling events; 4) sick pigs.
- Sequences from the surveillance programme will be deposited in GenBank.
- Survey design allows for investigations of human/animal health events.
- Samples will be anonymised to improve participation/cooperation of pig owners.

Gaps:

- Anonymous system limits the understanding of viral epidemiology.
- Swine influenza is not a program disease.
- Limited in scope and representation of all US swine populations.

Required resources:

- Isolates and antisera.
- Protocols and algorithms.
- Others as needed.

- **Canada** (*John Pasick*)

- SIV surveillance is not a nationally coordinated program.
- 60% of the hogs are raised in Eastern Canada (Quebec, Ontario) and 40% in Western Canada (Manitoba, Alberta, Saskatchewan, BC), there is more overlap with poultry in the East than in the West of Canada.
- In Manitoba and Alberta, influenza in swine is a notifiable disease, the feasibility of an active surveillance program is being considered.
- In Quebec, there is passive surveillance at the provincial laboratory and veterinary clinics.
- In Ontario, there is no active surveillance program in place but Ontario reports novel influenza virus isolates as part of research publications.
- Approximately 100 samples per month were tested for influenza in veterinary laboratories throughout Canada in 2009 and 2010.
- Submission of viruses to the CFIA Federal Laboratory to determine genetic characterization showed a lot of Cluster IV H3N2. 104 H1N1 viruses were analysed - 57 were collected prior to 2009, 47 after pandemic started - demonstrating all same alpha, beta, gamma, delta, and pandemic HA clusters of H1 as in the USA, the N1 were either pandemic, classical, or human-origin.

- **South America** (*Janice Ciacci-Zanella*)

- The National Program for Swine Health is concentrated on eradicating "major" diseases such as CSF, PRF, ASF, PRRSV, Brucella, TGE, VS; historically this has not covered influenza in pigs.
- There was sporadic detection of influenza in Brazilian pigs throughout the 1900s but no characterization was performed.
- In 2002 serological studies conducted and influenza antibody positive pigs were found.
- 2006 Mancini et al showed serological positive pigs - 85% to H1N1, 83% to H3N2, and 91% to flu B
- Current funding is targeted to detection of PRRSV, influenza, and PRV in pigs in Brazil.
- Virological detection of influenza in pigs includes H3N8 in 2008 in Toledo, State of Parana, via isolation and sequencing. Other influenza viruses found include pH1N1 2009. Epidemiological data on the H3N8 are lacking.
- The H3N8 influenza isolated from pigs was equine and similar to a 1963 lineage of A/eq/Miami/1/1963 H3N8. Affected pigs showed signs of pneumonia. Serological profiles of herds before and after pandemic showed an increase in antibody positive herds post-pandemic (using NP ELISA).
- Brazil would like to begin twinning with other laboratories. Brazil can serve as a reference laboratory in South America and provide training in influenza in swine to other South American countries because they have the necessary facilities, equipment and expertise.

- **Asia: Thailand** (*Sujira Parchariyanon*)

- There are several studies and surveillance activities on-going in Thailand on a national level (2000 to 2010) and several collaborative studies (NIH, AFRIMS, FAO, MOPH, OFFLU).
- Serological surveillance of 20,511 sera from 46 provinces was undertaken from 2003 to 2010 using HI tests identifying antibodies to 4 H1 viruses and 3 H3 viruses; mostly Thai isolates. Seroprevalence to H3N2 was higher than H1N1. Viruses were also isolated and were reassortants and of several genotypes.
- Central Thailand has both large poultry and pig farms, most of the farms are in good biosecurity. Samples from dense populations of pigs have yielded more viruses.

- **Asia: Japan (*Takehiko Saito*)**
  - Japan undertook passive surveillance in Japan in slaughter houses (MOH, starting in 1999) and on-farm (MAFF), resulting in a few isolates per year.
  - Characterization of H3 in Japan shows human to pig transmission. Human H3N2 has been found sporadically in Japanese pigs. H1 isolates are of several different lineages.
  - The Japanese undertook collaborative influenza surveillance programs in pigs in Thailand and Vietnam from 2005 and 2010, respectively.
    - o In Thailand, 1.9% of clinically health pigs - primarily young pigs – were positive for Virus Isolation (VI). Subtypes H1N1, H3N2 and pH1N1 2009 were isolated (20 VI positive of 1061 samples collected) - (Activity supported by J-GRID, MEXT, JAPAN).
    - o In Vietnam, on farm testing of serum and respiratory samples was undertaken both in the North and South, the VI positive rate was 0.9% with the same subtypes detected as in Thailand; characterization is pending. (Activity supported by MAFF, JAPAN).
  - Movement of pigs is likely from Vietnam to Lao and Cambodia (testing in Cambodia is undertaken by Institute Pasteur) and not the other way round.
  
- **Asia: Hong Kong (*Malik Peiris*)**
  - There are multiple introductions of influenza in pigs into China and evidence of inter-continental movement of virus lineages.
  - Influenza detected in pigs in Hong Kong up until 2000 was all classic H1N1, then introduction of European swine lineages in 2001 and TRIG US swine lineages in 2002, then continued detection of reassortment after that. Reassortment likely leads to genetic diversity and antigenic diversity.
  - It would be advantageous to consider expanding or re-focussing surveillance to include gene flow, and to combine surveillance with an assessment of the potential for transmission to humans (like ferret transmission, virus tropism in ex vivo human respiratory tract samples, human sero-epidemiology).
  
- **Australia & Oceania region (*Frank Wong*)**
  - Australia finally detected influenza in pigs in 2009 when the pandemic H1N1 strain went from humans to pigs.
  - If you think it's hard to keep developed countries interested in flu in pigs, try keeping developing countries interested.
  - The Australian international reference laboratory works in the SE Asian region for diagnostic capacity building and strengthening veterinary services for diseases such as HPAI, CSF, FMD, and PRRS.
  - In Vietnam and Thailand there are a lot of pigs, a significant proportion are reared in intensive commercial pig farming systems. In other SE Asian countries low biosecurity, back yard systems prevail.
  - AAHL-Indonesia collaborations for HPAI in poultry have resulted in characterization of approximately 300 H5N1 isolates from 2008 to 2010. The protocol for diagnosis follows an FAO RAP document and involves Flu PCR followed up by VI and characterisation.
  
- **NIH CEIRS global activities (*Richard Webby*)**
  - The current CEIRS influenza contracts run out 31 March 2014. There is discussion on whether this will be extended, so longer term sustainability of activities is an important issue.
  - Successes of the CEIRS programs to date include collecting and testing well over 5000 samples in Western Africa (all negative), detection of reassortant pandemic/endemic viruses in pigs in Hong Kong, Argentina, Thailand, and USA which is a reflection on the sensitivity of the testing plan and shows that surveillance is working.
  - CEIRS can contribute reagents, viruses' data, assay protocols, resources, and technical expertise.
  
- **CDC activities and priorities (*Ruben Donis*)**
  - The CDC mission is to mitigate the public health impact of pandemic influenza through early detection of viruses with novel HA and rapid development of effective vaccines.

- A main objective is on-going risk assessment to determine the pandemic potential of animal viruses through a standardized algorithm. Risk depends on viral characteristics, prevalence, global geographic distribution, host and environmental factors.
  - Risk assessment requires virological surveillance in swine herds with timely reporting and sharing of virus samples and data.
  - CDC approach is rooted in the one health concept domestically and internationally to enhance mutually beneficial influenza surveillance and timely reporting.
  - Support laboratory capacity building; provide training for risk assessment at the animal-human interface.
  - Gaps in availability of viruses from humans may exist because of increased reliance on PCR rather than culture for laboratory diagnosis.
  - Approximately 3,000-5,000 human influenza viruses are analysed each year by CDC. Due to the similarities in viruses circulating in pigs and humans, full genome sequencing is needed to find reassortants and “novel” viruses.
- **WHO perspectives and priorities** (*Liz Mumford*)
    - Establishing public health (PH) algorithms for characterizing unsubtypables is one goal. The WHO would like to know “what are the public health risks from influenza viruses of swine origin” and to figure that out needs epidemiological data, clinical data, and virological data. These data are needed for both animal and human viruses.
- **FAO activities and priorities** (*Filip Claes*)
    - Reporting of pandemic H1N1 2009 in pigs occurred mainly in the developed world and maybe that is due to a lack of capacity in developing countries, fear of trade implications, or focus on other diseases.
    - The FAO focussed its efforts on sampling in developing countries, for example, Costa Rica, Cuba, El Salvador, and Guatemala. This sampling determined a low level of pandemic H1N1 in pigs in Central America. Influenza surveillance was facilitated by combining with surveillance for other pig disease pathogens **including PRRSV, PCV2, Myco, CSF.**
    - A lot of hard work is necessary at the beginning to get the harmonization of protocols and sufficient diagnostic capacity building before moving ahead with the actual surveillance.
    - Countries are chosen for the FAO programme after expressing an interest in participating themselves and then they are enrolled.
    - FAO’s mission is to secure the food source of the people in the country.
- **OIE activities and priorities** (*Keith Hamilton*)
    - Influenza viruses in pigs that fulfil the criteria of a new and emerging disease are notifiable to OIE; this was the case with pandemic H1N1 2009 up until September 2010.
    - Although swine influenza is not an OIE listed disease it is considered to be an important disease and therefore there is a chapter on swine influenza in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals; this chapter outlines the international standards for detection and characterisation of SIV infections.
    - SIV is not an OIE listed disease and there should be no international trade restrictions following detection of influenza in pigs (including pandemic H1N1 2009) when there is no scientific justification to do so. There is therefore no chapter on SIV in the OIE Terrestrial Animal Health Code. Laboratories can apply to the OIE for OIE Reference Laboratory status for swine influenza if they are able to meet the mandate of an OIE Reference Laboratory.
    - Avian influenza surveillance through OFFLU and equine influenza surveillance through the expert surveillance panel are examples of two existing surveillance networks that are linked to OIE and that are sustainable. They both have a defined focus and meet annually.
    - There are the global strategy documents written by OFFLU that can and should be used as guidance when developing surveillance plans for influenza.
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- **Identifying regions for targeted surveillance** (*Lina Awada*)
  - A literature review of factors that are associated with influenza infection in pigs suggest that factors positively associated include importation of pigs, pig population density, number of animals on site, increased replacement rates, external source of breeding pigs, farms containing pigs kept indoors, and a variety of farm level factors like flooring type, farrow-to-finish type of farm, higher-parity sows, existence of open partitions between pens, uncontrolled entrance to the farm, ai/ao vs. cont flow, contact with other species.
  - Looking at pig movements and number of pigs, there are countries with a lot of pigs, and then those that import live pigs, and intensification level (e.g. more than 50% of the farms have >100 pigs per farm) and it turns out that all of North America, all of Europe (except the UK and Scandinavia), then Brazil and some countries from South-east Asia (China, Philippines, rep of Korea, Japan, Thailand). If you add in poultry, include all of SE Asia, east Europe (Poland, Romania, Russia, Ukraine) and some of West Africa (Nigeria). If you add in wild birds and/or surface water present, you get to add India. Looking at live pork exports will help perhaps determine the spread of the virus. Finally, when you add it all together, targeted surveillance should be done in China, Chinese Tai Pei, most of SE Asia, India, West Africa, Europe, North America, and Brazil.
- **Types of surveillance and cluster and geospatial analyses of SIV in the USA** (*Marie Gramer*)
  - Influenza surveillance for pigs in the United States comes from a variety of channels – active surveillance from research (from NIH CEIRS network funds), government sponsored passive surveillance that is voluntary, and syndromic surveillance from diagnostic case submissions to private and public (University) laboratories. Several clusters of H1 influenza – alpha, beta, gamma, delta, pandemic – and one predominant cluster of H3 influenza – cluster IV – co-circulate in U.S. pigs. The delta cluster is increasingly diverse. Recently the delta cluster sequences were shared with bioinformatics exports at the NIH. Because there was sufficient geographical and temporal data association with the delta cluster H1 sequences from pigs in the US, robust geospatial and temporal phylogenetic analyses could be completed. From these analyses it was concluded that these delta cluster (originally seasonal Human-origin H1) influenza viruses first emerged in the Southern US before disseminating into the Midwest, suggesting that the Southeast & South-central regions should be monitored closely for new virus introduction. These patterns of virus movement are driven by large-scale movements of swine from the South to Midwest. Rather than a conventional sink-source model, the spread of the virus flows from smaller to large pig populations, with the Midwest serving as a sink rather than source population. Therefore sharing of data and viruses in a confidential manner that manages to still protect the anonymity of the pork producer can result in significant findings and guide future influenza virus surveillance efforts.

**Day 2 – April 7, 2011**  
**DEFINING ACTION ITEMS AND OUTCOMES**

**Meeting outputs:**

1. Work towards establishing a worldwide surveillance network for influenza in pigs that includes timely data collection, analyses, and sharing.

2. Develop lists of viruses and appropriate diagnostic tests by region that is updated annually at each OFFLU meeting for guidance materials to be published on OFFLU website.
3. Harmonize laboratory methods
  - a. Step-wise harmonization may be necessary for labs of different capacity.
  - b. Update the OFFLU algorithm for testing for pandemic H1N1 in pigs to cover SIV  
[http://offlu.net/OFFLU%20Site/OFFLU\\_SIV\\_Surveillance\\_Testing\\_Algorithm.pdf](http://offlu.net/OFFLU%20Site/OFFLU_SIV_Surveillance_Testing_Algorithm.pdf)
  - c. Use the OIE manual, USDA manual, ESNIP as models for harmonized diagnostic testing methods
  - d. Consider developing a “kit” similar to the WHO model for HI antigens and antisera
  - e. Partnering with commercial companies with diagnostic kits to standardize assays
4. Need a statement on why the network is important in order to leverage funding and/or participation from swine producers.
  - a. Clarify the benefits of surveillance to the participating farmer or country
  - b. Need studies on impact of SIV to swine industry (meta data analysis of existing publications)
    - i. Economics
    - ii. Food security
    - iii. Animal health (burden of disease)
    - iv. Cost of pandemic e.g. testimony by Butler to US Congress
    - v. Vaccine usage data (CVB)
  - c. Improving vaccines
  - d. Improving diagnostic tests
  - e. Public health benefits
  - f. Identify funding sources for sustainability
5. Write a funding proposal to sustain group’s activity and fund targeted surveillance
6. Develop standard set of power point slides for meetings to promote the network
7. Write a manuscript using Lina’s data analysis as a framework for targeting surveillance globally

## Action Items

- Name the group (refer suggestions to OFFLU Steering Committee)
- Plan follow-up meeting in March 2012 (Kristien, Amy, Richard, and OFFLU).
- Press release or letter promoting the network’s activities (Keith)
- Circulate outputs, meeting discussion points, and presentations
- Contact meeting organizers (IPVS, AASV, ERPD, AAVLD, USAHA ISIRV) to ask for network member to present for promoting the network’s activities w/ standardized slides
  - Email Amy names of meetings and website link if possible
  - Develop communication for sending to meeting organizers (Keith, Amy, Kristien)
- Set up sub-committees for driving the priority outputs
  1. Publication of lists of viruses and diagnostic tests by region: Marie, Kristien , Malik, Sabrina, Janice
  2. Harmonization of lab protocols for influenza in pigs: Ian, Sabrina, Richard, Takehiko, Sujira, Frank
  3. Publication of network’s statement: Ruben, Ian, Liz, John
  4. Preparation of SI surveillance funding proposal: Kristien , Ruben, Filip, Richard
  5. Prepare of advocacy slide set about OFFLU SIV surveillance activity: Amy, Lina, Nicola
  6. Publish a paper on targeted surveillance : Lina, Kristien, Marie, Amy, Filip